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THE ECOLOGICAL DETERMINANTS OF POPULATION GROWTH IN A DROSOPHILA CULTURE

III. LARVAL AND PUPAL SURVIVAL[1]

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In our previous communication (Robertson and Sang, 1944) dealing with fecundity and prenatal mortality of *Drosophila* in artificial culture, the focus of inquiry was the role of the yeast organism as the food of the parent-flies. This paper deals with the relation of the yeast diet and other variables to the survival of larvae and pupae in such cultures.

Little is known of the significance of larval and pupal mortalities during *Drosophila* population growth. Harnly (1929) noted that in *Drosophila* cultures "maximum yields . . . are far below the population that might be produced if all the eggs resulted in flies"; but he gives no indication of the stage at which this mortality occurs or of its extent. Winsor (1937) suggested that the differences between the published data of Pearl (1932) and those of Pearl and Parker (1922) might be due to larval mortality produced by crowding of the larvae. This view finds support in the work of L'Héritier (1937) and from Bodenheimer's (1938) data on the total population count in a developing *Drosophila* culture. Indeed, this is to be expected, for larval crowding has been shown to increase mortality and delay the development of such different species as *Lucilia* (Salt, 1932), *Sitophilus* (Maclagan and Dunn, 1935), *Tribolium* (Park, 1938), *Callosobruchus* (Utida, 1941), and many others. But, if we are to understand the significance of these stages in population growth, we must know more than this. We must find what modifies the rate of larval and pupal development, the survival of these two stages, the relationship between rate of development and survival, and the significance of such changes for the development of a population.

When we turn to examine the circumstances which affect the larval and pupal stages of *Drosophila*, we find that the interaction between the two species pres-

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ent in the cultures (flies and yeasts) is supremely important. The yeast population grows at the expense of the nutrient substances present in the culture medium, and the larvae feed on the yeasts (Baumberger, 1919). We have to consider, therefore, the interaction between the two species as their populations develop. This is a very complex relationship. But we can get some information about its effect on the survival and development of the larval population by varying the ratio of initial yeast to initial larval bulk in two ways: (*a*) by crowding the larvae and (*b*) by increasing the yeast per unit larval mass.

EXPERIMENTAL

1. LARVAL CROWDING

Crowding of larvae is important because of its effects on: (*a*) numbers surviving to the pupal and adult stages, (*b*) the duration of the larval and pupal stages, and (*c*) the size and sex ratio of the resulting adults. Park (1938) uses larval mortality and rate of larval metamorphosis to appraise the "efficiency" of the entire larval period of *Tribolium*, i.e., points *a* and *b*, above. But selective mortality of the sexes and any change in size of resulting adults must also be considered. Both of these may modify mean duration of the stages as well as survival. If size decreases, the food required will be less; if the sex ratio is altered, differences in food requirements of the sexes may become important; and any change will certainly be of great significance in the future history of the population. Such a selective mortality of the sexes has been reported for *Lucilia* and *Theobaldia* (Herms, 1928), for *Tribolium* (Holdaway and Smith, 1933), and for *Lymantria* (Brandt, 1937).

The influence of larval crowding was examined in the experiments which follow. The methods for collecting larvae, etc., have already been described (Robertson and Sang, 1944). Larvae were removed from the oviposition medium within 3–6 hours of hatching and placed in varying numbers in 6 × 1-in. tubes containing 4 cc. of Pearl's S101 medium (Pearl and Penniman, 1926) solidified with 1.7 per cent agar. Pearl's medium was used because in other media it is difficult to see the pupae; this medium is transparent, so counts can easily be made without disturbing the contents. The tubes were seeded with 14 mg. of powdered brewer's yeast just before larvae were added. The numbers of pupae and adults were recorded daily, and flies were sexed and weighed within 6–10 hours of hatching. All experiments were run at 25 ± 1° C. The results of such an experiment are given in Table 1.

The technical procedure here, where we are dealing only with competition between larvae of the same age, differs from that of Park's (1938) experiments and from that of the experiments of Pearl and Parker (1922). Park kept the degree of crowding constant throughout his experiments by replacing all dead larvae by individuals from parallel cultures. Pearl and Parker allowed their flies to lay for several days, so that competition took place between larvae born at different times. Differences of egg viability are also excluded, since only living larvae are seeded onto the medium. By using the S101 medium we are sure that the yeast alone is the major source of larval food.

By inspection it was seen that two circumstances were changing as cultures developed. First, the quantity of yeast available at any time is determined by its growth, by redistribution by larvae which carry it into the medium, and by its rate of removal by larvae. When

crowding is great, the rapidity with which yeast is removed obscures the other changes. Second, the consistency of the medium is lowered by a mechanical breaking-up of the gel as larvae tunnel and plough through it. At great crowding it becomes almost completely fluid. These two changes are taking place throughout the whole larval period and to a varying degree, depending on the state of larval crowding. So far it has proved impossible to measure them.

be deleterious to the species, have been reported by Allee (1934). (iv) Total mortality is clearly made up of two parts—larval mortality and pupal mortality. That is, the previous history of the pupae influences their survival.

b) Larval and pupal mortalities.—The number of larvae dying is at first low, less than 10 per cent, and is roughly proportional to crowding, up to an initial value of 50 seeded. Deaths then increase exponentially until no larvae survive.

TABLE 1*

LARVAL AND PUPAL MORTALITY

No. of Larvae Seeded	No. Surviving		Percentage Mortality			Percentage of Formed Pupae Dying	Sex Ratio
	Pupae	Adults	Larval	Pupal	Total		
2.....	1.83	1.8	8.6	1.4	10.0	1.64	1.07
5.....	4.75	4.7	5.0	1.0	6.0	1.05	0.94
10.....	9.5	9.2	5.0	3.0	8.0	3.16	0.89
20.....	19.0	17.0	5.0	10.0	15.0	10.5	0.68
30.....	27.4	23.6	8.8	12.6	21.4	18.3	1.22
40.....	36.2	30.0	9.5	15.5	25.0	16.6	1.10
50.....	44.2	28.2	11.6	32.0	43.6	36.2	1.06
60.....	52.0	31.0	13.4	34.9	48.3	40.5	1.09
70.....	57.0	35.5	18.7	30.5	49.2	37.7	1.39
80.....	59.2	31.1	26.0	35.2	61.3	47.7	1.04
90.....	58.0	26.0	35.6	35.5	71.1	55.0	1.28
100.....	47.0	17.0	53.0	30.0	83.0	64.0	1.95
120.....	43.0	7.0	63.0	31.0	94.2	84.0	0.84

* Average results of 6 experiments. In each experiment 5 tubes were set up for larval density 2, 2 for density 5, and 1 for each of the other densities.

a) Total mortality.—Table 1 raises several important issues: (i) There is a low and more or less constant mortality even when crowding is slight. This mortality may be called "*minimal mortality.*" (ii) Only after crowding reaches a certain intensity does mortality increase, and this increase is roughly proportional to degree of crowding. (iii) When crowding is slight, there is an optimum at which survival is greatest. This optimum is not at the lowest degree of crowding under the conditions of this experiment. Similar optima, which show that undercrowding, as well as overcrowding, may

Thus crowding under these conditions has an extremely important effect on larval survival. This can be seen from the percentage larval mortality figures (Table 1).

The percentage increase of pupal mortality is proportional to crowding, up to an initial value of approximately 50 larvae seeded. After this it becomes constant. This apparent stabilization of pupal mortality is brought about by the real decrease in numbers of larvae reaching the pupal stage, which increases about this point. By examining the percentage of *formed* pupae which die, we find that

it is directly and consistently proportional to crowding (Table 1). Hence pupal survival also decreases, if there is crowding.

c) Duration of larval and pupal periods.—Crowding or starvation has long been known to prolong the larval period of insects, but few data exist relating this turning into small and only rarely viable pupae. The mean larval period increases geometrically with crowding within the limits examined. There is again a crowding optimum which is not the lowest value. There is a comparable optimum for variability, but at a higher degree of crowding; and there is remarkably little

TABLE 2

DURATION OF LARVAL PERIOD

Initial Larvae	Range (Days)		Mean (Days)	Standard Deviation (Days)	Coefficient of Variation (Per Cent)	No. of Larvae
	Max.	Min.				
2	6	4	4.95±0.115	0.637	12.80	30
5	5	4	4.70±0.100	0.533	11.34	29
10	5	4	4.80±0.098	0.471	9.82	29
20	5	5	5.00±0.055	0.418	8.37	58
30	7	4	5.26±0.074	0.556	10.57	57
40	7	5	5.40±0.047	0.560	10.38	144
50	8	5	5.70±0.065	0.755	13.24	134
60	9	5	6.40±0.072	0.886	13.84	152
70	11	5	6.60±0.089	1.160	17.57	170
80	10	5	7.16±0.096	0.954	13.32	98
90	11	5	7.54±0.100	0.997	13.22	100
100	12	6	8.20±0.111	1.109	13.52	101

TABLE 3

DURATION OF PUPAL PERIOD

	Range (Days)		Mean (Days)	Standard Deviation (Days)	Coefficient of Variation (Per Cent)	No. of Pupae
	Min.	Max.				
Uncrowded (2–20)	3	5	3.80±0.03	0.22	5.79	46
Overcrowded (70–100)	3	7	4.36±0.05	0.46	10.50	89

to population problems (Park, 1938). Indeed, no quantitative studies appear to have been made. From the experiments just quoted we can obtain some relevant information (Table 2).

The duration of the larval period is almost doubled under intense conditions of crowding; but this does not represent maximum possible prolongation of the larval stage. In experiments not here recorded, larvae lived for 14 days before increase of variability as crowding increases. The length of the larval period increases as mortality increases.

Since pupae were not isolated individually, the data given are incomplete. Among flies which emerge on any day, we are unable to distinguish those which have had a long preadult period because their pupal period is prolonged from those which may have had a long larval and a normal pupal period. Figures illus-

trating only the effects of crowding are given in Table 3. A high proportion of pupae formed under crowded conditions died, and all the pupae were small. In these circumstances, as Powsner (1935) points out, there is a high correlation between pupal and larval longevity. The increase of the pupal period is smaller than that of the corresponding larval period. Thus crowding increases the length of the preadult period primarily by prolonging the larval stage.

Even under optimal conditions, the length of larval life recorded here is greater than that found by Powsner (1935) and by Brehme (1938). This is probably due to the difference in food medium used. The writer's estimate of the length of the pupal period agrees closely with what these workers found.

TABLE 4

WEIGHT CHANGES WITH CROWDING

Initial Crowding	Weight (Mg.)	
	Male	Female
2	0.898	1.386
5	0.812	1.284
10	0.820	1.186
20	0.750	0.907
30	0.588	0.717
40	0.480	0.728
50	0.347	0.484
60	0.410	0.460
70	0.393	0.407
80	0.352	0.380
90	0.328	0.372
100	0.372	0.290
150	0.280	0.320

d) Weight of adults emerging.—It is also well known that overcrowded stocks of *Drosophila* produce undersized flies. The importance of this has not been recognized in population work. Table 4 shows that the weight of the female under optimal conditions is greater than that of the male. With increased crowding, the two weights decrease and become more alike until, under the most crowded conditions, they become the same. This lowest weight, which is approximately one-fourth the "normal," is the smallest possible survival size for both sexes. No smaller flies have been found in our experiments. The result also suggests that there is a simple relationship between male and female weights under these different conditions. And this is confirmed from a larger body of data which show a straight-line relationship between male and female weights.

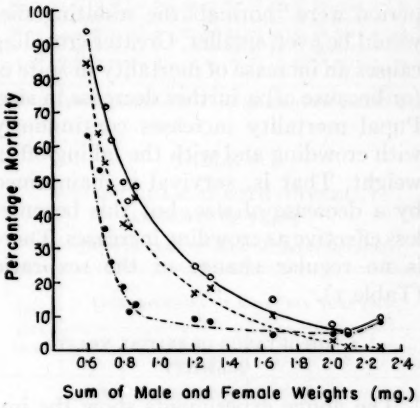

FIG. 1.—The relationship between adult weight and larval and pupal mortality. ○, preimaginal mortality; ●, larval mortality; *x*, percentage of formed pupae dying.

The fall in weight with increased crowding gives a regular curve, which comes to a constant value and is related logarithmically to the increase in mortality (Fig. 1). Larval mortality rises only slightly (less than 10 per cent) as weight decreases until the sum of the male and female weights has reached a value of 0.85 mg. Thereafter it increases rapidly. Pupal mortality (percentage of formed pupae dying), on the other hand, increases continuously as adult weight

falls. Larval and pupal periods both increase as weight decreases. These relationships are discussed below.

The changes brought about by increased crowding of larvae can be summarized as follows: In the larval period numbers are maintained at the expense of size and with an increase in duration of that stage. This increase may partly compensate for change in size, since it is reasonable to assume that if the larval period were "normal" the resulting flies would be even smaller. Greater crowding causes an increase of mortality in spite of (or because of) a further decrease in size. Pupal mortality increases continuously with crowding and with the falling-off in weight. That is, survival is maintained by a decrease of size, but this becomes less effective as crowding increases. There is no regular change of the sex ratio (Table 1).

2. IMPORTANCE OF INITIAL YEAST QUANTITY

The above experiments show the importance of preadult stages, and how crowding determines survival, rate of development, and size of the resulting adults. They do not show, however, whether these changes are brought about by crowding per se or whether they depend on available food. Nor do they indicate the parts played by the medium and by yeasts in the medium; that is, they do not tell us whether we can regard the medium as homogeneous or whether larvae play some part in yeast distribution and in this way determine the efficiency of medium utilization.

The importance of yeast and of the quantity initially available can be examined by seeding the tubes with different amounts. This procedure assumes that with an increase in initial yeast there will be a relative increase in its growth, as has been demonstrated by Hartelius (1934). The advantage thus gained should be apparent, whatever number of larvae is placed on the medium, up to a limit, even if the retention of this advantage is only for the initial period of larval growth. There should also be a limit to the effects of increasing yeast, since its growth is limited by the quantity of its nutrient material (the medium). If larvae assist or retard yeast growth, this should be apparent with changes of crowding. Again we have to emphasize that the breaking-up of the medium introduces a variable which cannot be estimated.

Tubes were prepared as for the previous experiment and were separated into 6 sets, each of which was seeded with a different quantity of live brewer's yeast. The numbers of larvae were varied in each set, and survival and weights were recorded. The findings for each tube are not always statistically adequate, but, as we are concerned only with the trend of results in each set, the number seeded, over 9,000, should give reliable information (Table 5).

a) *Preadult mortality.*—In general, no matter what the initial yeast quantity, crowding decreases survival. As before, there is evidence for a "minimal mortality" even under most favorable conditions. But this is not so definite as examination of any one set of data would suggest. The number dying, up to the point where mortality clearly begins to increase, decreases in proportion to the quantity of yeast seeded (12, 10, 8, 6, 7, and 4 per cent mortality, respectively). That is, the food quantity has a slight but significant effect even when there is little crowding.

When the initial yeast quantity is small, there is an optimal degree of crowding. This disappears when the

TABLE 5

MORTALITY WITH DIFFERENT INITIAL YEAST QUANTITIES (IN MG.)

No. Seeded	Per Cent Larval Mortality						Per Cent Formed Pupae Dying						Per Cent Total Mortality					
	7*	14	28	42	56	70	7	14	28	42	56	70	7	14	28	42	56	70
2	7.0	8.6	20.0	10.0	0.0	0.0	24.8	1.64	0.0	0.0	0.0	0.0	30.0	10.0	20.0	10.0	0.0	0.0
5	7.0	5.0	10.0	0.0	0.0	10.0	6.45	1.05	0.0	0.0	0.0	0.0	13.0	6.0	10.0	0.0	0.0	10.0
10	10.0	5.0	5.0	0.0	0.0	0.0	0.0	3.16	0.0	0.0	5.0	1.0	10.0	8.0	5.0	0.0	5.0	1.0
20	5.0	5.0	12.5	0.0	7.5	5.0	12.1	10.5	2.8	15.0	2.7	0.0	16.5	15.0	15.0	15.0	10.0	5.0
30	14.3	8.8	5.0	11.6	5.0	5.25	19.4	18.3	12.3	5.6	8.7	5.0	31.0	21.4	16.7	16.7	13.3	10.0
40	12.5	9.5	6.25	5.0	6.25	0.0	20.0	16.6	8.0	6.6	5.35	3.75	30.0	25.0	13.8	11.3	11.25	3.75
50	12.6	11.6	7.0	2.0	7.1	2.0	26.8	36.2	16.1	23.5	7.1	0.0	36.0	43.6	22.0	25.0	8.0	2.0
60	11.2	13.4	0.83	7.5	6.65	9.15	49.5	40.4	9.3	20.7	1.8	3.68	55.0	48.3	10.0	26.7	8.35	12.5
70	35.0	18.7	10.0	15.7	5.0	2.86	78.2	37.7	15.8	21.2	6.75	2.2	86.0	49.2	24.3	33.6	11.4	3.57
80	42.5	26.0	3.75	11.2	6.9	8.75	95.7	47.7	22.7	17.6	10.6	0.69	97.5	61.2	25.6	26.9	16.25	9.4
90	59.7	35.6	9.45	6.6	5.5	15.0	92.6	55.0	34.4	29.2	17.1	3.9	96.0	71.1	40.5	33.8	22.7	17.8
100	64.7	53.0	9.0	6.0	13.0	14.0	93.5	64.0	36.0	34.1	15.0	16.9	97.7	83.0	41.5	38.0	26.0	28.5
120	0.0	63.0	25.0	15.0	9.2	12.1	0.0	84.0	50.0	42.0	18.3	14.7	0.0	94.2	62.5	50.8	25.8	25.4
150	0.0	0.0	0.0	0.0	16.0	13.3	0.0	0.0	0.0	0.0	65.0	53.0	0.0	0.0	0.0	0.0	70.7	59.4

* The 6 sets of tubes were seeded with 7, 14, 28, 42, 56, and 70 mg. brewer's yeast, respectively. These quantities are used to designate the particular sets in this and subsequent tables.

yeast is increased. When there is little initial yeast, a sufficient number of larvae probably help to spread it through the medium and encourage its greater growth. A smaller number will not suffice to do so effectively; and a greater number will eat away the yeast more rapidly. With more initial yeast this spreading will become relatively ineffective. However, larval food requirements and yeast quantity will be altering daily, so a complex changing equilibrium must be present during each successive time interval. The secondary optimum which appears at greater crowding and with more yeast is not so striking and may be due to sampling errors. With the greatest yeast quantity, nearly all deaths take place in the pupal stage (Table 5).

The weight of emerging flies decreases with increased crowding, but the fall is less when the quantity of yeast is high (Table 6). With a maximum yeast quantity, crowding produces a significant effect even at low levels (Fig. 2). There is no evidence that the optimum for survival is reflected, as we might expect, by the weight of the flies. In the main, size reflects better survival conditions. The duration of the preadult period is increased by crowding and decreased by increase of initial food. This period, however, shows an optimum corresponding roughly to the survival optimum.

TABLE 6

WEIGHT CHANGES WITH DIFFERENT AMOUNTS OF YEAST

(Weight = Sum of ♂ and ♀ in milligrams)

Initial Nos.	Initial Amount of Yeast per Tube (Mg.)					
	7	14	28	42	56	70
2	2.38	2.284	2.34	.....	.....	.....
5	2.155	2.096	2.24	2.15	.....	.....
10	1.92	1.906	2.21	1.98	2.29	2.12
20	1.585	1.657	1.98	1.90	1.81	2.06
30	1.405	1.305	1.84	1.96	1.79	1.97
40	1.135	1.208	1.345	1.60	1.02	1.82
50	0.99	0.831	1.335	1.51	1.37	1.80
60	0.825	0.87	1.26	1.33	1.52	1.65
70	.....	0.80	1.03	1.27	1.38	1.585
80	.....	0.732	0.93	1.18	1.34	1.475
90	.....	0.70	0.75	1.08	1.29	1.49
100	.....	0.617	0.81	1.035	1.36	1.33
120	.....	0.60	0.79	.....	1.05	1.22
150	.....	.....	.....	.....	1.17	1.03

b) The relationship between yeast quantity and larval size and survival.—The numbers surviving and the weight of flies at different degrees of crowding on the various yeastings show a notable similarity in their changes in relation to

each other (Tables 5 and 6). This suggests some simple relationship underlying these changes. For example, the weight of emerging flies declines regularly with crowding, whatever the yeast quantity. By trial it was found that the

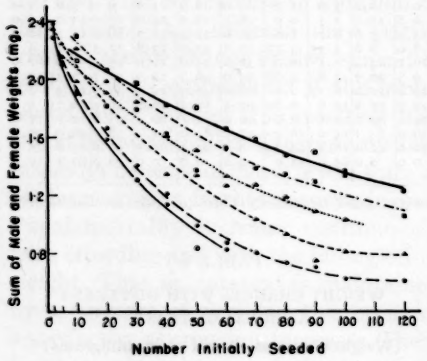

FIG. 2.—Changes of adult weight with crowding on media seeded with different initial yeast quantities. The weights are from a sample of 5 males and 5 females from each culture. ●, 7 mg. yeast; ○, 14 mg. yeast; ▲, 28 mg. yeast; △, 42 mg. yeast; ■, 56 mg. yeast; □, 70 mg. yeast initially.

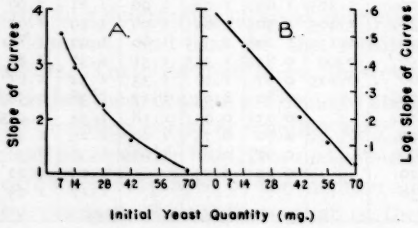

FIG. 3.—Changes of the slope of weight-crowding curves with different initial yeast quantities. *A*, slope of curves and yeast quantity; *B*, log slope of curves and yeast quantity (see text for details).

weight of the flies, in each case, had a rectilinear relationship with crowding when plotted semilogarithmically. That is, if w = weight of flies (sum of male and female weights), d = the initial degree of crowding, and k_1 and s are constants, then the relationship can be described by the empirical formula

$$w = k_1 e^{sd} . \qquad (1)$$

The constant k_1 is approximately the same for each yeasting within the limits examined, and the constant s, which gives the slope of the curves, varies with the quantity of yeast. Under these conditions, when the volume and area of the medium are constant, the largest flies turn up at the lowest degree of crowding, and such flies are of approximately the same weight. But, obviously, when we use smaller yeast quantities, we reach a level beyond which only smaller flies will emerge even under the most favorable crowding conditions. The constant k_1 will change at this point, and the relationship described will break down.

The constant s is a function of yeast quantity. Since increasing the yeast increases weight at any except the lowest level of crowding, it is not surprising that s is also an exponential function (Fig. 3). From this it follows that the slope of the curve will be zero when 140 mg. of yeast are initially seeded. That is, in this case, the weight of the flies will not be altered by crowding, up to the limits examined. Again we reach a point when crowding itself will begin to influence feeding, owing to interference of larvae with one another. The relationship stated will then become invalid. In nature such gross overcrowding will rarely occur, and limitation of food supply is a more probable cause of larval and pupal mortality.

Our data also show that mortality is related to body-weight. The percentage of total mortality is the only figure which gives a comparable value for all cultures. In relating it to body-weight (Fig. 4) we must note: (*a*) The histogram of mortality for all yeastings is regularly related

to weight, but at high weights the optimum found when there is little yeast decreases the regularity. As we are concerned only with general changes, this will be neglected. (*b*) Variability of weight is considerable, and flies emerging from a culture decrease in weight on successive days. Only the mean weight of flies on the day on which most emerged has been taken.

The errors of observation are sufficient to account for the spread of the points.

d = initial number of larvae, and k_1, k_2, k_3, c, and γ are constants. But $k_3 e^{\gamma k_1} = C$, a constant. Therefore,

$$m = C\, e^{dk_2 e^{cy}}. \qquad (3)$$

Both constants d and c are negative. If initial yeast (y) is kept constant as within a series or if crowding (d) is kept constant as between series, equation (3) becomes a simple exponential. For the following reasons this is true only within a limited range of y and d: (*a*) the optimum found

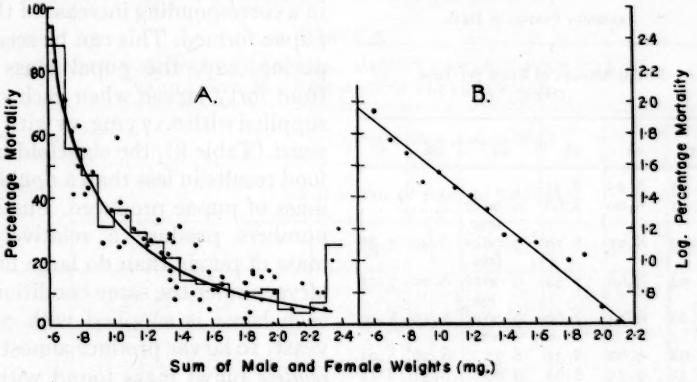

FIG. 4.—Preadult mortality and weight of flies emerging from cultures seeded with different quantities of yeast. *A*, histogram of mortality and weight changes; *B*, log mean percentage mortality and weight.

The solid line will therefore be taken to represent the general changes of mortality with weight. The percentage of mortality increases geometrically with decrease in adult weight (Fig. 4). So it can be described empirically by a formula similar to the first.

The complete empirical formula for changes in mortality and weight under the experimental conditions can be written as follows:

$$m = k_3 e^{\gamma k_1 e^{dk_2 e^{cy}}}, \qquad (2)$$

where m = per cent mortality, w = weight of flies, y = quantity of yeast, with little yeast and the relatively high mortality with ample yeast and low crowding and (*b*) the greater survival at high crowding. Both *a* and *b* invalidate the conclusion. The latter may be due to early larval mortality, releasing relatively more food for the few survivors. Even under specific and simple conditions, the mass of adults emerging is not related simply to crowding and available food.

c) *The preadult period.*—The length of the preadult period (larval and pupal instars) shows considerable variability, and it is difficult to interpret this in terms of crowding and food supply (Table 7). In

general, the duration of preadult life is proportional to percentage mortality, whatever the larval conditions. But there are irregular discrepancies within each and between series (see below). Similarly, the relationship to adult size is irregular, and, from what has already been stated, this is not surprising. Size decreases as the preadult period is prolonged. Hence extension of the larval (and following it, the pupal) stage permits greater growth of larvae and a correspondingly higher survival rate.

TABLE 7

DURATION OF PREADULT LIFE

Initial Crowding	Preadult Period in Days					
	Initial Amount of Yeast per Tube (Mg.)					
	17	14	28	42	56	70
2...	8.53	8.25	8.57	.....	.....	.....
5...	8.61	8.00	8.66	9.00 or less	.....	.....
10...	8.22	8.21	8.10	9.00 or less	8.40	7.88
20...	9.04	8.68	7.94	9.00 or less	8.00	7.95
30...	9.29	9.14	7.60	9.00 or less	8.10	8.00
40...	9.92	9.60	8.35	8.72	8.26	8.03
50...	10.51	9.25	8.68	9.09	8.35	8.12
60...	11.74	10.86	9.12	9.07	9.31	8.61
70...	11.07	9.78	10.17	9.37	9.13	8.71
80...	.....	10.8	9.72	10.41	9.35	8.55
90...	.....	11.00	10.18	11.18	9.65	8.77
100...	.....	.....	10.52	11.09	9.17	8.95
120...	.....	.....	10.00	11.79	10.21	8.84
150...	.....	.....	.....	.....	11.23	9.58

d) *Utilization of the medium.*—The ability of larvae to survive by prolongation of the stage and at subnormal sizes, even though this becomes progressively less efficient, is an indication of the complexity of the changes in a normal culture. By itself this gives little information concerning the importance of the yeasts in the medium. Unfortunately, it was not possible to measure the changes of the yeast population in these experiments. So we can examine only the indirect evidence relating to them, and for this we have selected the total mass of pupae formed in the vials (Table 8).

It is obvious that the medium does not become saturated with yeast, irrespective of the amount initially seeded: small quantities of yeast can support only a limited larval population. Nor does an increase of the initial yeast quota result in a corresponding increase of the mass of pupae formed. This can be seen by comparing, say, the pupal mass resulting from forty larvae when each of these is supplied with 0.175 mg. or with 1.4 mg. of yeast (Table 8); the eightfold increase of food results in less than a doubling of the mass of pupae produced. Further, small numbers produce a relatively greater mass of pupae than do large numbers of larvae under the same conditions (e.g., if each larva is supplied with 0.7 mg. of yeast, 10 larvae produce almost twice the *relative* pupal mass found with 100 larvae). In short, as judged by the quantity of yeast found by the larvae, small amounts of yeast grow relatively more efficiently, and small numbers of larvae interfere relatively less with yeast growth than do larger ones. Thus it is the relationship between initial yeast, larval numbers, and the amount of medium which determines the efficiency with which the last is utilized. The data are insufficient to show whether this is a consequence only of the searching behavior of the larvae in a limited space or whether the larvae do actively contribute to spreading the yeast through the medium. When there is an initial excess of yeast (more than 1.4 mg. per larva), crowding of larvae has little or no effect on the

mass of pupae formed within the limits examined here (Table 8).

When the quantity of medium is constant, changes brought about by differences of crowding and initial yeast quantity can be summarized as follows: Within the limits examined, (1) the weight of emerging flies decreases geometrically as the initial number of larvae is increased (this is true for all yeast quantities); (2) at any degree of crowding the weight of flies increases regularly when the initial yeast is increased; (3) the percentage of mortality increases exponentially as adult weight decreases, whatever the conditions of larval life; (4) with little yeast there is a variable optimum degree of crowding, suggesting some interaction between larvae and yeasts which stimulates growth of the latter; (5) increased crowding prolongs preadult life; (6) the effects of larval crowding depend on available food: there is no indication that they are produced by the direct actions of the larvae on one another; and (7) the initial quantity of yeast determines to some degree the efficient utilization of the medium. The complexity of this relatively simple ecological situation needs no further emphasis.

3. QUANTITY OF MEDIUM AND PREADULT CHANGES

While Pearl (1926), Harnly (1929), and others regarded the medium, the agar-gel, as the soil on which the adult's food (yeast) developed, they also assumed it to be the larval food. Thus Pearl says: "The larvae burrow about in and at the same time grow fat on the nourishing jelly." And again: "The adult flies will eat the yeast and the larvae will eat the banana-agar substratum." This is only partly true.

TABLE 8

RELATION BETWEEN THE INITIAL AMOUNT OF YEAST AVAILABLE TO EACH LARVA AND THE MASS OF PUPAE FORMED

Yeast per Larva (Mg.)	Total Pupal Mass Formed (Mg.)					
	Initial Amount of Yeast per Tube (Mg.)					
	7	14	28	42	56	70
2.8	.....	9.95 (5)	21.0 (10)	.....	33.5 (20)	.....
1.4	9.8 (5)*	18.1 (10)	34.6 (20)	52.0 (30)	68.0 (40)	88.3 (50)
0.7	21.3 (10)	31.5 (20)	55.0 (40)	74.0 (60)	100.0 (80)	114.5 (100)
0.35	30.0 (20)	43.7 (40)	71.6 (80)	.....	.....	.....
0.23	36.0 (30)	45.3 (60)	71.2 (120)	.....	.....	.....
0.175	39.8 (40)	43.4 (80)	.....	.....	.....	.....

* Initial numbers of larvae per tube shown in parentheses.

Observation of feeding larvae shows, as Baumberger (1919) first demonstrated, that they use their paired hooklike mouth parts to pull in parts of the medium and that this scooping movement continues when they are moving and feeding. Particles of yeast and medium torn off by this process pass into the gut. Probably larvae derive some nourishment from the medium itself when this is made with cornmeal and molasses or banana. This possibility was eliminated in these experiments by the use of Pearl's S101 medium, which, while it contains sugar, cannot supply any of the protein, etc., necessary for growth.

The quantity of medium available is

obviously important in relation to yeast growth and, therefore, to larval feeding. Pearl (1926) reports that an increase in the size of culture bottles from ½ to 1 pint, and therefore of the area of the food medium, gives an increased asymptotic population. This he claims is due to the increase in air space available to the adults. But the proof is not convincing (see Allee, 1931). Data to be presented later (Sang, 1949), in which quantity of medium is increased and *the air space decreased*, the experiments being conducted in ½-pint bottles, show that changes in quantity of medium alone can produce similar results.

Harnly (1929) found that depth, area, and consistency of medium all altered the relative survival values of wild type and vestigial flies and that they are therefore relevant environmental factors in selection. An increase of depth causes an increase in number of progeny of a single pair according to the law of diminishing returns. Similarly an increase of area increases the yield over an 18-day period. Two considerations here complicate the issue: (*a*) there is, as he shows, a competition between larvae of the two genotypes; and (*b*) numbers of larvae appearing in the cultures on successive days depend on the oviposition rate of the females. This is altered by available oviposition space, quantity of food, and other changes of the medium already described (Robertson and Sang, 1944).

Starting with an initial population of 4 pairs of flies, Bodenheimer (1938) finds that varying air space and volume of medium has no effect on maximum population in one series of experiments and "does not yield clean-cut results" in another. His data give only numbers of living flies found on any one day and not the yield of the cultures, i.e., total number hatching. Therefore, while his results contradict those of Pearl, they are not strictly comparable with those of Harnly or those of Gordon and Sang (1941).

In what follows, the importance of medium quantity has been examined by growing larvae under varying degrees of crowding and with the same initial yeast, on different area and depths of medium.

a) *Depth of medium.*—Larvae of *Drosophila*, cultured in vials, normally penetrate the medium to a depth of between 4 and 5 mm. This is as far down as they can reach without completely withdrawing their posterior spiracles from above the surface of the medium. In most vials some larvae penetrate to a greater depth, depending on the age of the larvae and the intensity of crowding. Apparently, they survive and feed normally even when their channels have closed over the spiracles. This can be for only short periods. In the main, larval feeding is therefore confined to a depth of about 0.5 cm. From this we should expect that an increase of depth would have little effect on larval survival and weight. But this is not so (Table 9). When crowding is great, some of the larvae crawl up the tubes, become dry and die.

In cultures of 350-sq. mm. surface area, increasing depth of medium increases the chance of survival of larvae when crowding is high, i.e., above the 40–50 level. This can never completely compensate for crowding, however deep the medium may be, since survival is considerably lower than when numbers are small. Depth has little effect on survival at low crowding. So depth effects are apparent only when initial crowding is great.

A similar picture is found for weight changes. Increasing depth raises the weight of emerging adults at all degrees of crowding but does so most markedly when the crowding is considerable. The

maximum size reached with 20 larvae appears to be greater than the maximum at 40, however deep the medium. This maximum is inversely proportional to initial crowding.

The mean duration of larval and preadult existence increases with increase of medium depth at low levels of crowding. The situation is not simple. There appears to be an optimum crowding level for a particular depth. Taken in conjunction with the data on survival and weight, this is the opposite of what we should anticipate from previous experiments. Hence the results (see below) relating to survival, size, and length of life are valid only under the particular conditions of these experiments.

TABLE 9

INFLUENCE OF MEDIUM DEPTH ON LARVAL CHANGES

Depth (Mm.)	Initial Crowding	No. Pupae	No. Adults	Mean Weight (Mg.)	Larval Period (Days)	Preadult Period (Days)	Per Cent Mortalty
5.5.....	20	19	19	1.27	4.17	8.26	5.0
	40	32	22	0.95	4.75	8.54	45.0
	60	37	17	0.57	5.11	8.77	71.6
	80	31	14	0.60	5.26	8.99	82.5
8.5.	20	13	12	1.97	4.46	8.09	40.0
	40	32	21	1.22	4.88	8.38	47.5
	60	33	24	0.85	5.12	9.03	60.0
	80	41	17	0.70	5.34	8.94	78.7
10.5.....	20	19	19	1.98	4.47	8.18	5.0
	40	34	29	1.55	5.09	8.92	27.5
	60	39	31	1.13	5.18	8.99	48.3
	80	53	21	0.83	5.46	9.24	73.7
17.0.....	20	19	18	2.02	5.05	8.99	10.0
	40	31	26	1.61	5.13	8.83	35.0
	60	48	36	1.25	5.54	9.34	40.0
	80	64	38	1.02	5.83	9.56	52.5

b) Area of medium.—Changing area of medium at constant depth will clearly have an effect on larval survival; for degree of crowding per unit area will decrease as the area is increased. At the same time, greater growth and spread of yeast over the surface will be possible. Consequently, we should anticipate an increase in yield and size and a decrease in preadult life when the area is increased. This is so (Table 10). The medium depth was kept constant at 1 cm. in these experiments. When there is a high number of larvae per unit area, the medium becomes fluid.

Considerable variability is found in the results. At a low crowding level, increased area has little influence on survival. The high mortality with 20 larvae on the smallest area occurred in the pupal stage and appeared to be due to a high percentage of larvae pupating in the medium and becoming overgrown with yeasts. This did not occur in the other tubes. At a higher level of crowding, increasing the area seeded decreases mortality. The weight of emerging flies increases with the area seeded, but at low crowding levels there is a limit. There is evidence for an optimal larval-area relationship when there are 60–80 larvae. The duration of the preadult period is af-

fected little at a low level of crowding. On the whole, it rises when crowding exceeds certain limits.

Repetition of these experiments confirmed the conclusions. They also show that survival increases noticeably until the area seeded is about 1,000 sq. mm. Beyond this there is little improvement. Meanwhile, the duration of the preadult period decreases up to a limit. The weight of the flies also increases. An even greater area than the largest tested would apparently yield larger flies.

TABLE 10

AREA OF MEDIUM AND LARVAL CHANGES

Area (Sq. Mm.)	Initial Nos.	No. Pupae	No. Adults	Mean Weight (Mg.)	Larval Period (Days)	Preadult Period (Days)	Per Cent Mortality
227	20	18	13	1.72	4.44	8.54	35.0
	40	33	28	1.32	4.36	8.68	30.0
	60	39	22	0.91	5.26	9.41	63.0
	80	38	12	.....	5.79	10.08	85.0
346	20	20	18	2.18	4.15	8.56	10.0
	40	34	31	1.98	4.03	8.19	22.5
	60	51	41	1.28	4.25	8.63	31.5
	80	64	59	1.07	5.66	10.79	26.0
530	20	19	18	2.13	4.(?)	8.50	5.0
	40	33	29	2.08	4.06	8.48	27.5
	60	54	45	1.69	4.20	8.58	25.0
	80	65	58	1.12	4.57	9.45	27.5
760	20	20	20	2.14	4.(?)	8.55	0.0
	40	37	33	2.00	4.11	8.73	17.5
	60	50	50	1.33	4.33	8.76	16.5
	80	64	50	0.91	4.63	8.88	37.5

c) Volume of medium.—In most previous work the volume of the medium is considered to be an adequate measure of its potential use by the larvae (Bodenheimer, 1938). But, as we have pointed out, few larvae feed beyond a certain depth. This suggests that area and depth effects are not the same even when the volume is constant. The relevant data from the preceding experiments (Tables 9 and 10) can be recalculated to show this. In spite of differences between experiments, we find that (1) volume changes have little influence on survival if the crowding is slight and (2) with intense crowding (e.g., 80 larvae), increased volume increases the yield. It apparently does so more effectively when area and not depth is increased. This difference between depth and area effects is confirmed by their different action on the duration of the preadult period.

d) Consistency of medium.—Larvae are able to burrow more readily in normal cornmeal-molasses cultures than in the "synthetic" medium, as the former medium does not tend to callapse on them so readily. Probably area and depth differences are not so marked in cornmeal media, but it is extremely difficult to determine the distribution of larvae in such cultures. Harnly (1929) shows that this factor, medium consistency, is important, and, by increasing the agar-agar from 0.7 to 3 per cent, he raised yields of bottle cultures by 46 per cent. Stiffer media tend to lower the yield. Also, pa-

per strips placed on the surface of 1 per cent agar medium brings about an even greater increase—up to 60 per cent. This is apparently brought about by older larvae working the paper into the medium. They thus give greater support to their tunnels and prevent the drowning of eggs and young larvae. So a more solid medium, within limits, increases the yield. We have been able to confirm the first of these results.

There is another side to this. Larvae fed on sterile synthetic media, which do not contain yeasts, grow most rapidly when the medium has a certain consistency (Fig. 5). Thus there is a consistency at which the gel can be most readily broken up and taken in by the larvae. This corresponds to optimal feeding conditions. Since the structure of the mouth parts changes during growth, this optimum is probably different for each of the three instars. Consistency, then, influences the growth and spreading of the yeasts, the support and feeding efficiency of the larvae, and the availability of the medium.

e) Conclusions.—The medium itself can influence larval survival, the duration of the preadult period, and the size of emerging adults. This is not due only to its consistency or hardness or to the quality or to the volume, though each of these is important, but apparently also to the depth and available surface area of medium. There is an optimum quality which depends on its suitability for yeast growth and an optimum consistency determined to some extent by the behavior characteristics of the larvae. Assuming these to be constant, changing the volume of medium by increasing depth increases survival, etc., when crowding is at a suboptimal level. Increasing the area has similar effects and under our experimental conditions is the more effective way of increasing volume.

Depth and area effects are not alike. Increasing the depth lengthens both larval and pupal stages, at the same time that it increases adult body-weight. Extending surface area, as found in the previous experiments (see also Harnly, 1929) increases body-weight but decreases duration of preadult stages. This difference suggested that it might be possible to distinguish how far duration of the pupal period depends on (*a*) the length of the larval period and (*b*) the ultimate size of the larva. No correlation

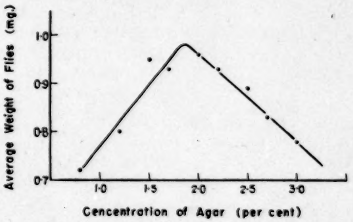

FIG. 5.—The effect of medium consistency on the adult weight of larvae cultured on a sterile synthetic medium.

was apparent. Therefore, duration of the pupal instar is not determined simply by pupal size or by length of the previous stage. It depends also on the *quality* of the food consumed. That is, specific food substances may be required for the production of growth-promoting hormones, etc. (see also Tatum, 1941). This suggests that Dover's (1931) view that metamorphosis is related to metabolism rather than to growth is correct and explains the variability of results shown in Table 7.

When recalculated, Harnly's (1929) data show that he obtained a greater yield per volume of medium by increasing its depth, not its area. The differences in experimental technique are important here. Harnly allowed a single pair of flies

to lay in bottles until the first adults hatched, then he counted and removed the flies hatching each day. His results indicate that the deep media gave "a reserve supply of food which carried through development those larvae hatching from eggs produced during the latter part of the 10-day period of egg-laying." The data are inadequate to show whether, when these are discounted, this difference is still found.

4. LARVAL CROWDING IN STERILE MEDIA

In the cultures used in the preceding experiments the yeast grows at the expense of the medium in the tubes, and the quantity present at a given time is a complex function of its growth rate, the speed at which it is consumed by the larvae, and changes in the physicochemical state of the medium. Consequently, the amount available to a larva at a given time will depend on the previous history of the culture. The above results show that there is a more or less regular sequence of events in such cultures and that these can be described, if somewhat crudely, by a simple mathematical formula. They do not tell us the part played by the yeasts in the medium or whether the secretions and excretions of the two species modify larval survival and growth. These two points can be tested by culturing larvae on a nongrowing medium, i.e., in sterile cultures. Only the importance of larval metabolic products will be examined here.

TABLE 11

LARVAL CROWDING IN STERILE SAWDUST-YEAST MEDIUM

Larvae per Vial	Per Cent Mortality	Developmental Period (Days)	Adult Weight ♂+♀ (Mg.)	No. Emerging
50.....	23.0	9.69±0.06	2.04	154
100.....	28.0	10.31±0.06	1.60	142
125.....	36.5	10.10±0.06	1.38	238
150.....	44.6	10.84±0.07	1.18	83
175.....	41.5	10.87±0.06	1.20	307
200.....	51.8	10.79±0.08	0.90	289
225.....	57.3	11.08±0.09	0.78	96
250.....	54.8	11.21±0.07	0.68	113
275.....	72.1	11.43±0.05	0.67	229
300.....	81.2	11.69±0.09	0.60	169

Sterile yeast-agar cultures were found to be unsatisfactory for our purpose, and the technique given by Gordon and Sang (1941) was used instead. One improvement was introduced into their procedure: the sterilizing fluid was made more effective by doubling its mercuric chloride content. The use of a sawdust-yeast medium meant that these cultures differed from live yeast cultures in two respects: (*a*) the yeast is not growing, so the quantity available to the larvae decreases as cultures age; (*b*) the medium is not a gel but a "paste" and is, therefore, different in physical structure; in particular, it is not so readily broken down by the feeding movements of the maggots. Because of this second difference the sterile experiments are not comparable with those which have gone before, and we can draw conclusions only concerning the effect of larval excretory products.

Two series of experiments were set up. In the first, different numbers of larvae were crowded in 0.5 gm. of medium (1 part brewer's yeast to 5 parts sawdust) moistened with Pearl's S101 salt solution containing 3 per cent dextrose. In the second, a constant number of larvae (50) was seeded onto the same medium containing different amounts of yeast. The results are given in Tables 11 and 12.

As we should expect, larvae crowded in sterile media have a greater mortality, longer developmental period, and smaller size than uncrowded larvae. The relationship between crowding and weight of emerging adults is, in this case, the same

as that found for live yeast cultures, i.e., it is semilogarithmic and corresponds to that found for cultures seeded with 70 mg. live yeast. But the size-mortality relation is not the same in the two cases; there is considerable difference between the two groups of experiments. In our sterile cultures pupal mortality is lower than on live yeast. It is difficult accurately to estimate pupal mortality in sawdust media, but one series of counts gave values below 5 per cent for cultures containing 200 larvae. The greatest mortality is, therefore, during the larval stage. Further, at low crowding, up to 175 larvae per tube, the mortality at corresponding weights is higher than on live yeast. With more intense crowding this difference disappears. The length of pre-

TABLE 12*

EFFECT OF VARYING YEAST CONTENT OF SAWDUST MEDIUM

Yeast per Vial (Gm.)	Per Cent Mortality	Developmental Period (Days)	Adult Weight ♂+♀ (Mg.)	No. Counted
0.10.....	21.3	9.98±0.05	2.00	118
0.08.....	34.6	10.73±0.11	1.88	98
0.06.....	37.0	11.89±0.08	1.34	126
0.04.....	49.3	13.30±0.12	0.94	76
0.02.....	85.3	15.82±0.25	0.76	22

* Total medium = 0.5 gm., miostened with salt solution in proportion of 6 cc. per gram sawdust per vial. Fifty larvae seeded in each culture.

adult life is again increased with crowding. It is always greater in sterile than in live media, except when the flies are very small. Changes of the preadult period correspond to changes of adult weight. Three differences between sterile and live yeast cultures can account for the results enumerated in this paragraph: (1) the quantity of yeast present in the two types of cultures will not be the same at different stages of larval development; (2) the quality of the yeasts remains more or less constant in sterile cultures but probably changes in live yeast cultures; and (3) the availability of yeasts and the support offered by the medium will be different in the two cases.

When available yeast is reduced in sterile cultures, survival is lowered and development impeded (Table 12), and the general picture is again similar to that found for live yeast media. But mortality and duration of preadult life are

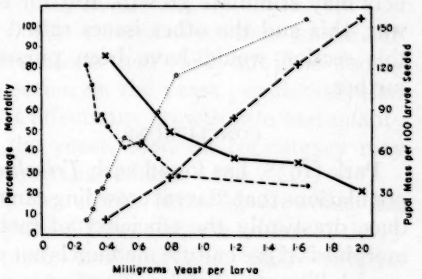

FIG. 6.—Changes in the development and survival of larvae cultured in sterile media containing different initial yeast quantities per larva. Heavy lines give the results from cultures in which the yeast is reduced and the number of larvae kept constant; light lines from cultures with a fixed yeast quantity and variable numbers of larvae (see text).

not related to adult weight as they are in live yeast cultures. Figure 6 shows that less than 0.3 mg. of yeast per larva is insufficient to support population growth. If we discount this minimum requirement, then the mass of pupae formed is directly proportional to the quantity of yeast in the tubes.

In the first sterile culture experiment there is a greater number of larvae present in the medium for any initial quantity of yeast per larva than in the second experiment. Contrary to expectation, we find that this increases the rate of development, lowers mortality, and raises the weight of flies which emerge from these cultures (Fig. 6). However, when there is very little yeast (less than 0.5 mg.) or

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more than 1.8 mg. yeast available to each larva, the two groups become alike. This is not a simple situation, but it suggests that larval excretory products, etc., do not discourage larval development to any great extent. The difference between experiments is probably due to the fact that the greater number of larvae mix the medium more thoroughly and so make it more accessible to the population. It is also possible that larval metabolic products may stimulate growth. But for the war, this and the other issues raised in this section would have been pursued further.

CONCLUSIONS

Park (1938) has found with *Tribolium* populations that "larval crowding can reduce drastically the efficiency of metamorphosis if the culture medium is not renewed. The essential density effect is not the behaviouristic interaction between organisms but represents rather the cumulative influence these organisms exert on their environment." This general statement is also true for *Drosophila* populations. But, in this case, the environment is a complex, of which one part, the yeasts, is another changing population. While we have shown that the quantity and quality of the other environmental component (the medium) influences "the efficiency of metamorphosis," we have suggested that it does so, for the most part, by its action on the yeast population. It affects the quality as well as the growth rate of the yeasts. The development of the yeast population also depends on the quantity of yeast initially seeded and on the numbers of larvae developing in the cultures; that is, there is an interaction between the two developing populations of organisms. This interaction is more or less regular in form, within certain limits, and the size of the initial populations is important in this connection.

When the quantity of yeast available to the larvae is small, their survival is lowered, development prolonged, and size diminished. According to Park (1941), a similar result has been reported by Vladimerova and Smirnov (1938), who used populations of *Musca* and *Phornia*. With certain initial yeast populations, undercrowding as well as overcrowding is detrimental to larval survival. This is already a well-known phenomenon (Allee, 1941), but the explanation of it appears to be different from that suggested by Allee (1931) in his analysis of Eigenbrodt's (1925) data for *Drosophila*. This optimum apparently depends on the balance between (*a*) the normal growth of the yeast and (*b*) its removal and its distribution through the medium by the larvae. Such an optimum has not been found for insect larvae which live in a nongrowing medium, for example, *Tribolium* and *Callosobruchus*, and we have been unable to obtain the result with *Drosophila* larvae cultured in sterile media. Sterile cultures also show that larval metabolic products do not impede, but may even encourage, larval development. "Conditioning" of the medium is due, therefore, only to a reduction of its nutritive value. Preliminary experiments suggest that this is also the case for *Tribolium* larvae cultured in flour (Sang and Begg, unpublished).

In the normal *Drosophila* bottle culture, larvae hatching from the eggs laid on any day will compete for food with larvae from other batches of eggs. This competition will certainly affect their development and survival and may do so up to the limits found in our tube cultures. But, as has been seen, the relationship is not rigidly fixed, and in bottle cultures it will depend on the quantity and

quality of the food available to the maggots at the different stages of their development. For this reason we must emphasize that experiments of the type presented here do not give us information which can be *directly* applied to population growth; they tell us only about the mass physiology of the fly and delimit the variables which may be important during population growth. However, these changes of larval survival and also of duration of preadult life must invalidate such theoretical calculations as those of Bodenheimer (1938).

SUMMARY

An analysis of the effects produced by crowding *Drosophila* larvae has given the following data:

1. Numbers are maintained at the expense of size and by a prolongation of preadult life. This becomes less effective as crowding is increased. The preadult developmental period may be trebled, and this increase takes place mainly during the larval stage. Also, the flies which emerge from very crowded cultures may be one-fourth the "normal" size; and preimaginal mortality may be 100 per cent.

2. The relationship between initial yeast quantity and developmental changes induced by crowding is more or less constant. The quantity of yeast determines, in part, the efficiency of medium utilization. Undercrowding as well as overcrowding may be detrimental to larval survival.

3. The volume of medium in the cultures affects their yield, but it does so by its action on the yeast population; that is, it affects the growth rate and quality of the yeast. Medium consistency may have similar effects.

4. In sterile cultures, crowding has slightly different effects on larval development and survival. Such cultures show, too, that larval metabolic products do not interfere with larval survival.

5. The significance of these changes in the mass physiology of the fly are discussed in the light of population theory.

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THE ECOLOGICAL DETERMINANTS OF POPULATION GROWTH IN A DROSOPHILA CULTURE

IV. THE SIGNIFICANCE OF SUCCESSIVE BATCHES OF LARVAE[1]

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LITTLE has been done to investigate the importance of larval mortality during the growth of insect populations. While the researches of Winsor (1937), Bodenheimer (1938), Park (1938), Utida (1941), and others suggest that larval and pupal mortality may be of importance, they do not show at which stage in the history of a population this mortality comes to play a decisive part in regulating its growth. We have shown that in the normal *Drosophila* culture preadult mortality depends on the quantity and quality of food (yeast) available to the larvae and that starvation prolongs the preadult period (Sang, 1949*a*). In such cultures, therefore, the population growth rate will change in response to alterations of the larval nutritional environment, and that environment will be modified by the activity of the larvae themselves. Unfortunately, it has proved impossible to measure the day-to-day changes of the yeast population in the *Drosophila* bottle. So we can only attempt (*a*) to determine whether larval mortality and prolongation of the preadult period do contribute significantly to the course of population growth and (*b*) to measure the extent of this contribution if there is one. Using the information obtained in our previous work (Sang, 1949*a*), we may be able to form an estimate from such data of the changes of the yeast population. The experiments should also tell us whether Winsor's (1937) suggestion that the difference be-

[1] The writer wishes to express his thanks to Dr. C. Gordon and to Miss J. M. Macdonald for permission to analyze data (Table 1) collected jointly with them in another connection.

tween the data of Pearl and Parker (1922) and of Pearl (1932) is due to larval mortality.

EXPERIMENTAL

1. THE FATE OF SUCCESSIVE BATCHES OF LARVAE

We have shown that the parent flies present in population cultures lay different numbers of eggs on successive days (Robertson and Sang, 1944). This difference in the size of the daily larval batch necessarily complicates any experimental analysis of larval changes during population growth. For example, it is difficult to draw any conclusion, other than that larval mortality may affect the later stages of growth, from Bodenheimer's (1938) data, which give the numbers of eggs, larvae, pupae, and adults present on each day in the life of a culture. Consequently, to simplify the analysis, the same number of larvae must be seeded onto the medium each day. This procedure will not duplicate the conditions found in a normal culture; but, if we vary the size of larval batch, we can obtain a reasonable estimate of these conditions, as well as a measure of the action of the larvae in the cultures. Larvae of the mutant "Antennaless" were used in the experiments which follow, so the data are comparable with those given by Gordon and Sang (1941).

Various-sized batches of "Antennaless" larvae were placed in sets of milk-bottle cultures of Pearl's S101 (modified) medium (see Robertson and Sang, 1944) for a period of 8 days (Table 1). The results show that, when the number of larvae seeded each day is small (10), the yield is more or less constant throughout the whole emergence period. When the number seeded is great (160), the yield decreases rapidly as cultures age. With 80 and 160 larvae seeded each day, the greatest yield is on the first day; lower seedings (10, 20, and 40) give a higher yield on the second day. In all cultures there is a secondary increase of yield on the sixth to seventh days, as well as other subsidiary increases. The productivity

TABLE 1

SURVIVAL AND WEIGHT CHANGES OF LARVAE SEEDED ON SUCCESSIVE DAYS

	Average No. Flies Emerging					Weight (Mean Male/Female) (Mg.)				
No. larvae seeded...	10	20	40	80	160	10	20	40	80	160
No. cultures.....	8	6	4	2	2	8	6	4	2	2
Day of emergence:										
1.....	5.6	9.0	22.5	59	83	1.20	1.19	1.13	1.07	0.87
2.....	7.3	16.0	28.0	43	61	1.05	1.15	1.18	0.92	0.67
3.....	3.3	10.9	16.3	6	22	1.13	1.14	1.12	0.83	0.52
4.....	4.5	9.0	17.0	25	9	1.12	1.08	0.96	0.55	0.45
5.....	3.9	13.7	12.0	19	5	1.12	1.10	0.86	0.52	0.56
6.....	7.9	18.3	20.0	4	9	1.07	1.06	0.69	0.63	0.57
7.....	2.8	4.5	13.0	11	12	0.95	0.95	0.63	0.63	0.60
8.....	0.4	.....	6.2	11	4	.....	.....	0.60	0.48	.....
9.....	.....	.....	6.2	1	1	.....	.....	0.59	.....	.....
10.....	.....	.....	4.0	.....	.....	.....	.....	0.74	.....	.....
Total yield.....	35.7	81.4	145.2	179	206	.....	.....	.....	.....	.....
Per cent survival...	44.6	50.8	45.4	28.0	16.1	.....	.....	.....	.....	.....

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of the cultures is increased when the number of larvae seeded is increased, up to a limit (Fig. 1). But the proportion of larvae surviving to the adult stage decreases as the size of the larval batch is increased, except for the lowest seeding

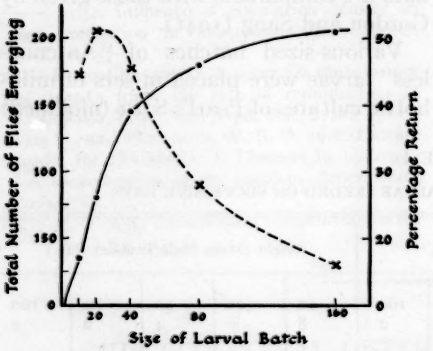

FIG. 1.—Yield of cultures seeded with different numbers of larvae; each culture received the same size of larval batch on successive days. Solid line gives the yield of cultures; broken line shows percentage survival.

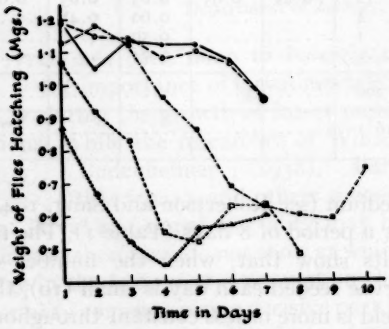

FIG. 2.—Weight changes of flies emerging from cultures seeded with different larval batches. ● = 10 larvae; ○ = 20 larvae; ■ = 40 larvae; □ = 80 larvae; ▲ = 160 larvae in each daily batch.

(10). This again suggests that, under these conditions, there is an optimal larval concentration which is not the lowest degree of crowding (Fig. 1). The percentage of larvae surviving is inversely proportional to the size of the batch seeded when these are of more than 20 larvae, and this percentage follows the law of diminishing returns.

Differences in weight of the emerging flies show that even flies emerging on the first day are influenced by crowding (Table 1), the weights being proportional to the number of larvae seeded. In general, weights of flies emerging on successive days decline as the cultures age. This fall of weight is greater when the number of larvae is high (Fig. 2). However, there is later a rise in weight of flies emerging from the more crowded cultures. This rise, which does not correspond to the sixth-to-seventh-day increase of yield, appears earlier in the more crowded cultures. Thus competition between larvae of different age groups influences survival, weight of emerging flies, and, consequently, the length of the preadult stage. The effects of this competition are not so simple as our experiments on larval crowding might have led us to expect (Sang, 1949*a*).

We should anticipate that the quantity of yeast available to the larvae would decrease from day to day or would remain more or less constant after an initial decrease, for we are placing the same number of larvae on the medium daily. This is partly confirmed by weight changes. But the increase of yield on the second and sixth days and increased weight of flies emerging during the later stages of culture invalidate this hypothesis. These latter facts imply at least two crises occurring during the development of the cultures: one about the second day of culture, the other about the sixth. Such crises may be due to changes of the quantitative relations between larval and yeast populations or to qualitative changes in the yeast population or to both. The earlier appearance of the rise of adult weight when cultures are more

crowded suggests that at least one such crisis is produced by a qualitative change. And this change must be produced by larval activity.

Repetition of this experiment using normal cornmeal-molasses in place of Pearl's S101 (modified) medium confirmed these general conclusions. It also showed that the course of events is not precisely the same in these two types of culture. The subsidiary emergence peak (sixth to seventh day) appears about 1 day earlier, and the productivity of the cultures is about 40 per cent greater when the cornmeal medium is used. Also, the weight of the flies emerging on any day is usually higher, and the secondary rise in weight takes place later in these cultures. That is, cornmeal-molasses is better than Pearl's synthetic medium, and the changes which occur during population growth do so more slowly in it than in the latter medium. The difference between the two media is shown in a further experiment (Table 2).

TABLE 2*

COMPARISON OF LARVAL SURVIVAL ON PEARL'S S101 MEDIUM AND ON CORNMEAL-MOLASSES MEDIUM

Day	No. Emerging		Mean Weight of Flies (Mg.)	
	Ser. A	Ser. B	Ser. A	Ser. B
1.....	36.8	42.4	2.13	2.15
2.....	34.4	23.8	2.17	2.10
3.....	14.6	16.4	2.04	1.97
4.....	16.2	20.4	1.95	1.84
5.....	6.0	4.2	1.82	1.81
6.....	5.2	18.2	1.73	1.75
7.....	5.4	8.8	1.54	1.72
8.....	6.8	9.0	1.49	1.66
9.....	4.6	12.4	1.03	1.66
10.....	4.2	13.8	1.09	1.57
11.....	4.8	17.0	1.01	1.46
12.....	2.4	10.8	1.11	1.56

* Series A: Pearl's medium; Series B: cornmeal medium. Sixty larvae seeded daily for 11 days in both series. Each result is the mean of 5 cultures.

2. "ANTENNALESS"-EBONY EXPERIMENTS

To examine the possibilities outlined above and to clarify further the competition between different larval age groups, successive daily batches of larvae have to be isolated within a population, and isolated in such a way that they can be readily identified. This is easily accomplished by growing the experimental batch in a mutant population; a procedure used earlier and in another context by Gordon and Sang (1941). The mutant, *ebony*, easily recognized as an adult, was the one chosen. Unfortunately, no easily recognizable larval mutants are available. So it is not possible to follow changes at this stage.

Normal culture bottles were separated into groups of ten. Each bottle of the first group received 80 larvae ("Antennaless") on the first day and 80 ebony larvae on each succeeding day. The bottles of the second group were seeded with 80 ebony larvae on the first day, 80 "Antennaless" on the second, and 80 ebony on the following days. The third group was seeded with "Antennaless" larvae on the third day and ebony on the preceding and succeeding days. And so on. The culture bottles were incubated for 24 hours before use to allow for the period of the egg instar. The results of this experiment are shown in Table 3 and in Figure 3. They should be compared with a similar experiment by Gordon and Sang (1941).

While the initial larval seeding of these experiments in no way duplicates the course of a normal culture containing one pair of adults, the total number of flies hatching approximates closely in the two situations. Thus, while the emergence curve is different (see Sang, 1949*b*), the total changes can be considered as roughly similar. Adults do not appear in con-

stant numbers each day. Hence there may be a change in the duration of the preadult stage or an increase in mortality. While mortality varies, there is no considerable decline in percentage survival with increase in age of cultures. However, there is a marked change in the duration of the preadult period. On the average, it increases directly with the age of the culture at the time of counting. There is, too, a great increase of variability. This can be seen from the spread of the results. These conclusions are also true of the experiment performed by Gordon and Sang (1941).

A repetition of the experiment with the use of only 40 larvae per day gave a yield of 206 flies per bottle. The results (Table 4), though again irregular, confirmed previous findings. In all three experiments increase of the preadult period from the first to the last day of the count

TABLE 3

SURVIVAL AND EMERGENCE OF SUCCESSIVE BATCHES OF LARVAE

Day of Emergence	Day of Seeding							No. Emerging	Duration of Life (Days)
	1	2	3	4	5	6	7		
1	59.6	.....	.....	.....	.....	.....	.....	59.6	10.0
2	1.66	44.22	.....	.....	.....	.....	.....	45.88	10.04
3		18.33	3	.....	.....	.....	.....	21.33	10.86
4		1.33	22.11	.....	.....	.....	.....	23.44	11.06
5			35.33	1.8	.....	.....	.....	37.13	11.96
6			5.0	8.8	.....	.....	.....	13.8	12.36
7			1.55	28.7	21.22	0.1	.....	51.7	12.61
8				7.5	32.22	1.11	.....	40.83	13.16
9				.5	10.33	14.9	.....	25.73	13.44
10					1.44	18.11	6.22	25.77	.....
13						13.0	23.88	36.88	.....
Daily total	61.26	63.88	66.99	47.30	65.21	47.13	30.10	.....	.....
Duration (days)	10.03	10.33	11.70	12.92	12.88	.....	.....	.....	.....
Per cent survival	76	80	84	59	81	59	?	.....	.....

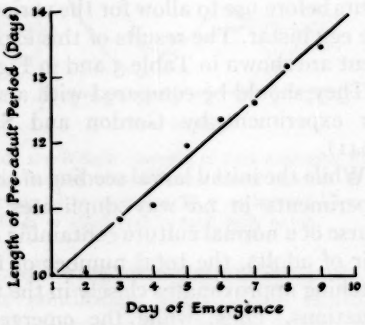

FIG. 3.—Length of the preadult period of flies emerging on any day from "Antennaless"-ebony cultures. (Data from Gordon and Sang, 1941.)

is greater than 3 days. Larvae seeded on the fifth day again develop more rapidly than those seeded on the fourth day. But the prolongation of the preadult period is not so great as in cultures seeded with 80 larvae. Since larvae present on and after the fifth day of culture will live at more or less the same level of crowding, subsequent competition is apparently due to changes in the yeast population.

Yeast population growth can be modified by the use of a different medium (Robertson and Sang, 1944). Fifty larvae were placed, as in the previous experiments, in bottles made up with Pearl's

S101 medium (modified). Unfortunately, a new sample of agar was used, and this set more firmly than usual. So larval feeding was considerably hindered. Nonetheless, certain interesting points appear. The 10-day count is very low, and the percentage mortality is consequently high, increasing daily after an initial stable period (Table 5). Duration of the preadult period increases from day to day, as in the previous experiments, and is again proportional to the age of the culture. This increase takes place more rapidly because culture conditions are bad.

The yield on any day is higher for the ebony than for the "Antennaless" mutant. The difference is slight and remains practically constant. For the entire emergence period 1.2 ebony flies hatch for every "Antennaless" fly. Thus the ebony mutant has a higher survival value under these conditions. This will make little difference to the results found for "Antennaless" flies. The mean weight of male and female ebony flies hatching on any day is always higher than that of the "Antennaless" flies. The weight of both mutants decreases as the cultures grow older (Table 5). The weight on the first day is normal, but it falls somewhat more rapidly than in cultures started with flies. Weights are related to length of preadult period approximately as in our previous larval crowding experiments (Sang, 1949*a*). But the remarkable rise in weight of flies hatching between the eighth and eleventh days is again found.

TABLE 4*

SURVIVAL AND LENGTH OF PREADULT LIFE OF SUCCESSIVE BATCHES OF LARVAE

	Day Seeded					
	1	2	3	4	5	6
Per cent survival....	87	92	88	76	96	70
Life-duration (days).	10.1	10.1	10.9	12.46	12.19	11.7

	Day of Emergence								
	1	2	3	4	5	6	7	8	9
No. emerging.....	32.0	36.8	10.4	23.0	13.8	23.4	40.2	24.0	2.8
Developmental period (days).....	10.0	10.07	10.27	11.03	11.36	11.95	11.79	12.32	13.21

* 40 larvae seeded each day on normal culture media.

DISCUSSION AND CONCLUSIONS

We are now in a position to re-examine the significance of (*a*) the increase of yield on the second and on the sixth to seventh days and (*b*) the increase of weight of flies emerging during the later stages of the cultures. Our data and those of Gordon and Sang (1941) show that the batch of larvae seeded onto the medium on the second day always has a slightly higher survival rate than the batch seeded on the first day (Tables 3 and 4), and this is increased when the batch is small. The second-day batch, however, takes slightly longer to emerge than does the

first-day batch. This increase of the preadult period is greater when the size of the batch is increased. Hence, when the number seeded is small, we have a higher emergence on the second than on the first day (Tables 1 and 4). If the batch is a large one, the prolongation of the preadult period will obliterate this rise. In the same way the increased emergence on placed in the medium on the fifth and sixth days. Such larvae tend to take longer to develop and have a higher mortality than do larvae of the preceding batches. So the change of the yeast population which produces this rise of flyweight is probably a qualitative one. This is also suggested by the data given in Tables 1 and 2.

TABLE 5

EMERGENCE OF "ANTENNALESS" AND EBONY FLIES ON PEARL'S S101 (MODIFIED) MEDIUM

Day of Emergence	"Antennaless" Group (4 Bottles)					Mean Emergence	Mean Weight (♂+♀) (Mg.)	Mean Life (Days)	Ebony Group (16 Bottles)					Mean Emergence	Mean Weight (♂+♀) (Mg.)
	1	2	3	4	5				1	2	3	4	5		
1	3	.....				0.75	.....	9+	...	...	...	...	...	0	.....
2	102	3				26.25	2.35	9.95	1	162	81	143	113	31.25	2.48
3	16	85				25.25	2.25	10.17	148	9	172	83	92	31.5	2.35
4		32	66			24.5	2.03	10.34	133	86	37	94	126	29.75	2.09
5		3	46	14	1	16.0	1.73	10.79	59	47	23	51	67	15.44	1.82
6		1	8	15	.....	6.0	1.27	11.41	30	54	53	26	63	14.75	1.45
7				9	2	2.75	1.26	11.82	42	29	33	12	30	9.11	1.38
8				10	4	3.5	1.50	12.72	15	17	10	3	2	2.94	1.42
9	6			9	17	8	1.27	13.1	21	27	18	14	3	5.18	1.39
10	10			6	19	8.75	1.45	13.87	17	31	34	18	9	6.81	1.48
11	12			3	28	10.75	0.98	14.79	27	37	52	29	40	11.56	1.40
12	3	1		4	2	2.5	0.92	15.9?	20	29	46	38	34	10.43	1.16
13	2	4	1	1	2	2.5	0.90	15.8?	10	9	28	17	17	5.06	1.05
Total	121	124	120	71	75	137.5	.....	.....	534	537	587	528	596	173.8	.....
Mean life duration (days)	10.12 13.54	10.32 16(?)	10.53	12.57	14.03										

the sixth or seventh day is seen to be brought about by a higher survival of the larval batch seeded on the fifth day and to a slight decrease of the preadult period of this batch (Tables 3 and 4). Both increases may be produced by a quantitative or qualitative change of the yeast population.

The increase in weight of emerging flies appears about the seventh or eighth day of emergence in one experiment (Table 5). These flies have a preadult period of approximately 12 days. That is, the majority have developed from larvae

Since the two groups of experiments (Tables 3 and 4 and Table 5) are not strictly comparable, we cannot determine whether the change of weight and the sixth-day increase of yield are produced by the same crisis in the culture medium. The late appearance of the increase of yield in Table 5 suggests that they are independent of each other. So does the lack of correspondence between the two when the size of the batches is increased (Table 1). But the effective medium change may take place at any time during the larval instar and influence,

let us say, the size of one batch and the survival of quite another batch of larvae. This is improbable, and it is more likely that two crises occur during the development of the cultures. However, we cannot be certain of this until we obtain more information about the changes which take place in the yeast population.

From the above discussion we see also that the relationships found between larval survival, length of preadult period, and weight of adult flies when only one batch of larvae is grown in a culture (Sang, 1949*a*) are not obtained when successive batches are seeded. Competition between batches and the action of the larvae on the quantity and quality of the yeasts, and on the structure of the medium, modify the relationships. This confirms our previous finding, namely, that there is no strict relationship between larval development and mortality.

Bodenheimer's (1938) data show that during the first 10 days of population growth the parent-flies lay an average of 80–90 eggs per day (range 12–290) and that preadult mortality, excluding egg inviability, is about 73 per cent. This experiment was performed in cultures containing Pearl's S101 medium, and the result is in close agreement with the figure given in Table 1. In our experiments, crowding increases the mortality only of larvae seeded on and after the fourth day, while the larval period of those seeded after the second to third day is prolonged. Probably similar changes are also found in population cultures. If this is so, then both increased time of development and preadult mortality influence the growth of the population. Unfortunately, the experiments of Pearl and Parker (1922) and of Pearl (1932) are not comparable; but again the preadult mortalities recorded here correspond closely to those suggested by Winsor (1937) in his analysis of their work. So adult crowding affects the productivity of their cultures not only by modifying the fecundity of the parents but also by producing a more intense crowding and, therefore, greater mortality among the larvae.

SUMMARY

While it is known that crowding alters the development and survival of *Drosophila* larvae, there is no information relating this to population growth. This paper is concerned with preadult mortality, etc., as they affect the successive batches of larvae which develop in population cultures. The data presented show that, in general, larvae which appear late in the life of a culture are smaller, take longer to develop, and have a higher mortality than those which appear first. This competition is intensified when the size of the larval batches is increased; and the details of these changes have been worked out by isolating the experimental batch in a mutant population. The experiments suggest, too, that there are two crises during the first 10 days in the life of a culture—one which affects larvae seeded on the fifth day and is more or less stable, and another the appearance of which depends on the degree of larval crowding. These crises may be produced by quantitative or qualitative changes in the yeast populations. Prolongation of the development of larvae seeded on and after the second day and the higher mortality of larvae seeded on and after the fourth day will both modify the course of population growth in *Drosophila* cultures.

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THE ECOLOGICAL DETERMINANTS OF POPULATION GROWTH IN A DROSOPHILA CULTURE

V. THE ADULT POPULATION COUNT[1]

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IN THE first paper of this series (Robertson and Sang, 1944) the writers pointed out that previous workers on population growth in the *Drosophila* yeast system have drawn conclusions mainly from counts of numbers of adults emerging from cultures. For example, such a count provided the experimental basis for Pearl's (1926) formulation of the "logistic law" of population growth. He fitted a logistic curve to the numbers of live adults found in his culture bottles from the first to the twenty-eighth day. More recently Bodenheimer (1938) applied the same curve to the numbers of adults found in his cultures during the first 15 days. He also fitted it to the total population (eggs, larvae, pupae, and adults) found during the first 21 days. But the egg-adult period of *Drosophila*, under these experimental conditions, is about 10 days. So the second generation of flies will not lay eggs until the eleventh day, and these eggs will not develop into flies until the twenty-second day at the earliest, and probably not until the twenty-fifth day. All flies which hatch before this time will be the progeny of the parents used to start the cultures. Hence both workers have applied the law to what is essentially a phase of "family growth" and not to true population growth.

These and other analyses have been made without regard to direct observation of changes of fecundity, preadult

[1] The writer is indebted to Dr. Cecil Gordon for permission to use data collected jointly with him, and for his encouragement and advice. Thanks are also due to Professor Lancelot Hogben, F.R.S., who supervised the work and criticized the manuscript.

mortality, or length of preadult life as the population develops. We have already seen that these changes are important and that neglect of them has led to some confusion (Sang, 1949*a*). They are of prime importance during family growth. In this communication we will consider how far the numbers of adults found during this phase can be taken as an index of changes of parental fertility and of preadult life under different environmental conditions. We shall also examine the relation of adult mortality to the environment. Data collected by Gordon and Sang (1941) will be extensively drawn on for this purpose.

EXPERIMENTAL

Pearl (1926) started his cultures with different numbers of parent-flies—in some cases adult flies and immature stages; in others, single virgin pairs. This is justifiable on the assumption that similar results are obtained "if one pair or a small population of *Drosophila* is introduced into milk bottles." This assumption is not correct when we are dealing only with family growth. But to safeguard against accidental failure of cultures through death of parents, it is convenient to start cultures with three pairs of 3-day-old flies. It is also convenient to remove and kill the offspring each day. Both procedures are here used in certain experiments in which we are not concerned with the course of family growth or mortality of adults but with the influence of environmental changes. When this is not so, the cultures are seeded with single virgin pairs.

The culture media, etc., employed have already been described (Gordon and

TABLE 1

DAILY EMERGENCE PER BOTTLE ON NORMAL MEDIUM

Day	Experiment No.		
	1	2	3
1	55.8	73.2	53.7
2	56.4	59.0	80.3
3	46.5	80.2	67.0
4	31.6	60.9	61.7
5	23.3	54.1	30.1
6	31.2	67.4	45.1
7	29.7	51.1	30.6
8	16.0	38.7	18.3
9	13.8	28.4	16.7
10	8.0	37.1	25.1
Total	312.3	550.1	428.7
No. bottles	16	7	6

TABLE 2

VARIABILITY OF ADULT EMERGENCE ON NORMAL MEDIUM

Day	Total	Mean	σ	Coefficient of Variation (Per Cent)	Females / Total
1	893	55.81	17.30	31.0	0.52
2	903	56.44	14.07	24.9	0.48
3	744	46.50	11.50	24.7	0.52
4	505	31.56	12.70	40.2	0.47
5	373	23.31	7.27	31.1	0.52
6	499	31.19	11.38	36.5	0.51
7	475	29.70	11.52	38.7	0.47
8	257	16.01	8.53	53.2	0.48
9	221	13.81	9.18	66.4	0.45
10	127	8.00	6.00	75.0	0.52
10-day total	5,045	315.31	73.96	23.5	0.49

Sang, 1941; Robertson and Sang, 1944). We shall first consider changes of daily emergence in 3×3 cultures and how these reflect various experimental situations.

1. DAILY EMERGENCE CHANGES

Changes of daily emergence in normal cornmeal-molasses-agar medium containing 0.8 per cent Nipagin and seeded with 2 drops of 2 per cent Hutchison's yeast are given in Table 1. The 10-day yield in these three experiments varies considerably. This difference is noticeable even on the first day. In all three experiments set out in this table, the greatest frequency of emergence occurs on the second and third days. Following this, there is a more or less regular decline of productivity, with a secondary peak on the sixth day. Variability within a single experiment and daily changes of the sex ratio are given in Table 2. These data are from the experiment recorded in the first row of Table 1.

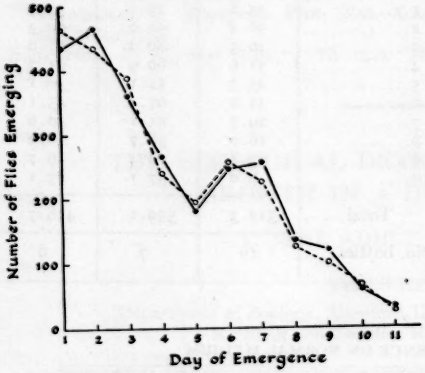

FIG. 1.—Daily emergence of flies from three-pair cultures. ●, number of males, and ○, number of females, hatching each day.

Daily sex-ratio changes show a slight but not very regular variation. More females than males emerge on the first, third, fifth, and sixth days; but this makes very little difference to the form of the emergence-time graph (Fig. 1). Hence differences due possibly to sex differences in the length of the preadult period (Bonnier, 1926) can be neglected when we are considering only changes in the trend of the emergence-time graph. The sex ratio for the entire 10-day period is 1.02 males to 1 female as compared with 6 females to 4 males found by Bodenheimer (1938) and 0.97 males to 1 female recorded by Eloff (1940).

The variability of yield changes daily (Table 2). It is lowest on the second and third days (25 per cent) and increases significantly up to the tenth day (75 per cent). Examination of counts for individual cultures shows that this is due to the rapid falling-off of some, while the yield keeps above the average in others. The detailed figures show that a low 10-day yield (total) is correlated with a low yield on the first, on the first 2 days, and still more during the first 4 days. Rank correlations are $r = 0.54$, 0.46, and 0.64, respectively. While considerable variability is apparent during the first 2 days of emergence, cultures which show a low yield at this stage generally produce fewer flies than the average.

Since the emergence graph (Fig. 1) is based on the average for all bottles, its level must be determined by the proportion of good and bad cultures. Nonetheless, the highest yield is attained on the first or second day in all bottles. Cultures with low yield come later than those with high. A sixth- to seventh-day peak is always present. It appears earlier in poor than in good cultures. This suggests that yield is altered by (*a*) the presence of parents of low fecundity or the early death of some of the flies in the culture; (*b*) specific culture conditions, e.g., poor yeast growth; or (*c*) both, for the two may be related.

2. CLASSIFICATION OF CULTURES

The successive batches of larvae which hatch from eggs laid by the parents develop under different environmental conditions. Gordon and Sang (1941) have drawn attention to these differences of culture conditions in relation to the exhibition of the gene "Antennaless." Daily examination of cultures shows that a regular series of changes takes place. On the first 2 days the yeast seeded grows rapidly on the surface of the medium, and only a small number of first-instar larvae can be seen crawling over this. On the third and fourth days larvae begin to penetrate the medium. As a consequence, the surface becomes pitted. By the fifth and sixth days there is a considerable variability in appearance of cultures, but in most cases the medium is full of channels produced by feeding larvae of all stages. The yeast is now no longer on the surface only; it has spread and has been carried into the medium. There is a slight smell of acetic acid and alcohol. From the eighth to the tenth days cultures differ markedly. Some are plowed up, so that the surface is pulped and wet. Others are still dry and full of air channels, while some show varying quantities of mold on the surface. On the tenth day the first adults appear. The classification of cultures outlined below was adopted because the tenth day showed these differences in appearance to the greatest degree. Of three classifications tried, it alone gave any regular result (see Gordon and Sang, 1941).

Four groups of cultures were set up. Two contained the antimold Nipagin. The bottles were seeded with either 2 per cent Hutchison's (wet baker's) yeast or ½ per cent Perolin (dry baker's) yeast. These concentrations gave comparable cell counts. The four groups are indicated by the following initials. H = Hutchison's without Nipagin, NH = Hutchison's with Nipagin, P = Perolin without Nipagin, NP = Perolin with Nipagin. On the tenth day the cultures of each group were arranged in five arbitrary classes based on appearance: Class I, cultures very wet and pulpy; Class II, cultures wet; Class III, dry cultures; Class IV, cultures showing slight mold; Class V, very moldy cultures.

The data in Table 3 show that the number of bottles in each class varies considerably. Consequently, it is impossible to calculate adequate statistics for each class. Taking group H, Class I (18 bottles), as a sample of variability within each group, we find its total variability for the 10-day yield (coefficient of variation = 11.42 per cent) is half as great as that of the unclassified cultures (Table 2). This means that our procedure does group similar cultures together. Variability in this case does not increase steadily as the cultures grow older, but on the first day the coefficient of variability is as high in the selected as in the unselected group. Hence declining yield of poor cultures must begin after the third day, as suggested by the rank correlations.

The duration of the cultures, estimated by the number of days during which they produce 10 or more flies, is greatest in all groups (H, NH, P, or NP) of Class I and tends to be least in Class V. Total yields over 10 days decrease from Class I to Class V in the order indicated by the Roman numeral. The only culture which falls out of line is NP I. This trend begins to be manifest on the second day and is well established by the fourth day. The peak yield also decreases from Class I to Class V (Fig. 2). Emergence is usually highest on the second day, but the decline which follows is modified by subsidiary peaks which appear capriciously

on the fourth, sixth, and eighth days. These latter show no regular change from class to class or between groups.

The antimold Nipagin causes a decrease of the number of cultures in Class I in both H and P groups; that is, contrary to expectation, it increases the number of bad and molded cultures. It also decreases the emergence period of the cultures. On the other hand, it increases the daily yield of cultures in all classes for both yeast (H and P). This striking effect on emergence is apparent as early as the second day, being more notable in Classes IV and V. Probably Nipagin influences emergence by its action on the yeast. This supposition is corroborated by the findings of Gordon and Sang (1941), who have shown that Nipagin influences the exhibition of the

TABLE 3

CLASSIFICATION OF CULTURES (FROM GORDON AND SANG)

Group	Classes				
	I	II	III	IV	V
	No. of Bottles				
H	18	2	3	2	2
NH	3	5	9	6	5
P	7	5	13	2	0
NP	4	8	7	4	6
	Duration of Emergence (Days)				
H	15	14	12	10	11
NH	12	12	11	11	11
P	15	14	12	6	0
NP	12	12	10	9	6
	Nos. Emerging				
H 0– 2 days	136	134	109	60	66
H 0– 4	286	281	219	107	131
H 0– 7	416	424	301	157	171
H 0–10	525	510	356	207	220
NH 0– 2 days	143	133	130	128	130
NH 0– 4	297	297	271	237	220
NH 0– 7	474	475	376	340	301
NH 0–10	609	611	465	421	372
P 0– 2 days	99	89	82	37	.....
P 0– 4	247	212	160	110	.....
P 0– 7	407	343	236	152	.....
P 0–10	494	425	299	162	.....
NP 0– 2 days	99	92	89	85	66
NP 0– 4	199	204	206	167	117
NP 0– 7	316	348	329	255	148
NP 0–10	398	435	393	284	158

mutant "Antennaless" indirectly through the yeasts.

The two yeast groups show that the 10-day yield of all classes is higher for Hutchison's than for Perolin yeast. This is true for initial numbers emerging (i.e., second day), for the level of the highest emergence, and throughout the life of the culture (Table 3). *Hence high initial larval population does not necessarily lead to subsequent lowering of yield*, as we might expect from experiments on larval crowding (Sang, 1949*a*). The addition of Nipagin does not alter this relationship, so it must have a more or less regular effect. Time-emergence graphs are slightly different for the two yeasts, Perolin tending to retain its peak from the second to the fourth days. The type of yeast makes little if any difference to the probability of producing cultures of one or another class: but the data are insufficient to allow detailed examination of this.

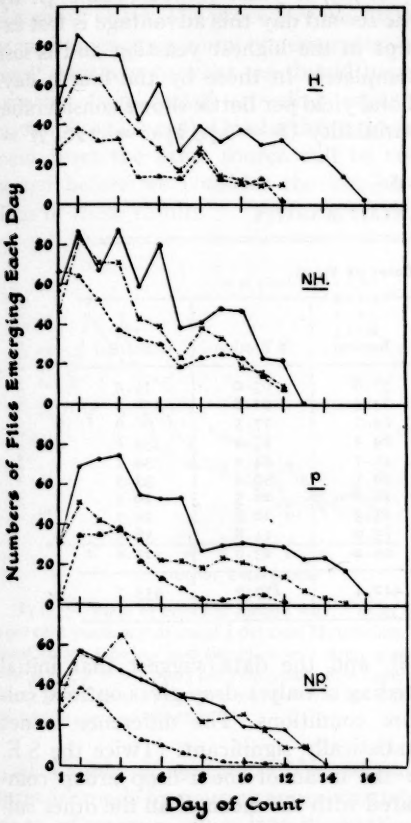

FIG. 2.—Yields of cultures of different classes and of different groups. Only Classes I, III, and V are shown for each group. Class IV is given in place of Class V in group P. ● = Class I; X = Class III; △ = Class IV; ▲ = Class V.

TABLE 4*

YIELD WITH BREWER'S YEAST

DAY	NO. EMERGING	
	Series 1	Series 2
1	4.1	19.3
2	17.3	22.7
3	11.2	26.4
4	17.5	34.5
5	21.7	38.7
6	26.0	26.2
7	30.8	34.1
8	21.2	22.6
9	18.4	26.1
10	18.3	24.4
11	11.6	21.4
Total	198.1	286.4

* Mean values from seventeen cultures in each series. Series 1, brewer's yeast seeded onto normal medium containing Nipagin; series 2, the same without Nipagin.

Since the type of yeast is important in connection with yield, it is worth recording the results obtained with the same media seeded with brewer's yeast (Table 4). With these the yield is very low, and the course of emergence different from that already found. In this case Nipagin *lowers* the yield of the cultures.

3. CONDITIONS OF CULTURE

The above finding confirms the statement that qualitative differences of yeasts initially seeded have a considerable influence on yield of cultures. Such dif-

ferences may involve the actual constituents of the yeast cells; but we have to reckon with another possibility. Since an initially vigorous yeast growth might well encourage a dense initial larval population which might seed the yeast more effectively through the medium and thus maintain the maximal level, the significant difference between different brands of yeast may be due to the rate at which the microörganisms propagate themselves. Such an interaction between larson's yeast. Three pairs of 3-day-old "Antennaless" flies were introduced into each bottle. Cultures which showed molds and yielded no flies after the sixth day were excluded from the totals.

Initially, the yield is proportional to the quantity of yeast seeded (Table 5). By the second day this advantage is lost except in the highest yeasting and is lost completely in those by the fourth day. Total yield per bottle shows considerable variability ($\bar{x} = 479.2$; $\sigma = 126$; $N =$

TABLE 5

INFLUENCE OF INITIAL YEAST QUANTITY

Day	No. Drops of Yeast				
	1 (9 Bottles)	2 (6 Bottles)	4 (9 Bottles)	8 (8 Bottles)	16 (6 Bottles)
1	54.6	56.9	57.8	65.0	79.5
2	86.7	85.0	83.4	91.8	100.2
3	75.2	72.8	64.7	77.5	66.6
4	63.7	68.8	64.2	57.4	39.1
5	47.2	34.8	41.7	44.4	34.7
6	65.1	50.2	38.1	39.4	33.3
7	37.1	32.8	26.8	28.5	20.3
8	39.8	19.7	21.3	20.6	21.7
9	35.6	19.0	25.0	21.8	36.5
10	26.3	29.2	19.4	23.6	21.8
Total	531.3	471.1	442.4	479.7	453.7

vae and yeasts can account for the observed class differences. It may also explain the group differences (H, NH, P, NP). No regular sequence that excludes such a possibility is apparent when we arrange the cultures in order of yield as follows: NH I, NH II, H I, H II, P I, NH III, NP II, P II, NH IV, NP III, NP I, NH V, H III, P III, NP IV, H V, P IV, NP V.

a) *Initial yeast quantity.*—To probe more thoroughly the possibility that different brands of yeasts have different initial growth rates, cultures of normal medium with Nipagin were set up and seeded with 1–16 drops of 2 per cent Hutchi-38), and the data suggest that initial seeding of only 1 drop gives optimal culture conditions. The difference is not statistically significant. (Twice the S.E. of the mean of the 1-drop group compared with the mean of all the other cultures—since they are roughly constant—gave a value of 96, greater than the difference of the means, 77). *Therefore, the initial quantity of yeast has little effect on yield, and quantitative differences cannot alone be responsible for the results found in previous experiments.*

This conclusion is further confirmed by data of Gordon and Sang (1941). The same quantity of yeast was seeded in 1 or

8 drops, spread over the medium. It was assumed that spreading the inoculum would increase the initial growth rate, as with bacteria (Stephenson, 1930). Two experiments show (Fig. 3) that, while there are slight differences within and between experiments, such differences are of a minor order. The procedure has little or no influence on numbers emerging, a result contrary to what we should have expected from larval crowding experiments (Sang, 1949*a*). One further experiment from the same source will be reported before we consider the implications of these results.

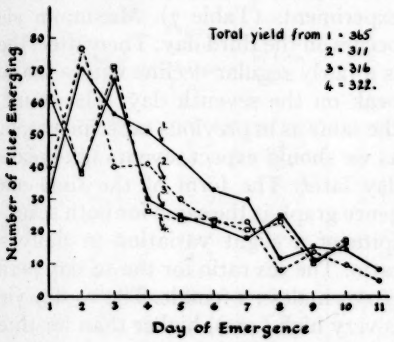

FIG. 3.—Yield of cultures seeded with 1 drop of 4 per cent yeast or 8 drops of ½ per cent Hutchinson's yeast (from Gordon and Sang). *1* = 1 drop 4 per cent yeast; *2* = 8 drops ½ per cent yeast, first experiment; *3* = 1 drop 4 per cent; and *4* = 8 drops ½ per cent yeast, second experiment.

b) *Volume of medium.*—Normal cultures are made by pouring approximately 50 cc. of warm medium into the bottles. No precautions are taken to insure accurate quantitative measurement. Two sets, one containing 25 cc. and the other 100 cc. of medium, were prepared, to examine the importance of this. A notable difference was found in the two series (Table 6) both in daily and in total yields. Daily emergence remains almost parallel. This remarkable result suggests that the difference is not due to a more rapid development of the larvae in the deep cultures but to higher fertility of the females. The relationship involved, however, must be a complex one.

By including the results of the previous experiment (Table 2) for the 50-cc. medium, we see that increasing the medium increases the yield regularly: the

TABLE 6*

VOLUME OF CULTURE MEDIUM AND YIELD

Day	Yield per Bottle	
	25-Cc. Medium	100-Cc. Medium
1	12.8	20
2	42.2	66.6
3	36	51.8
4	47.4	63.2
5	16.8	27.2
6	20.7	42.4
7	20.2	35.6
8	14.7	26.7
9	17.7	25.8
10	14.6	22.6
11	9.3	15.7
Total	252.4	397.6

* Six cultures were used for each series.

curve approximates the logarithmic type. This confirms Harnly's (1929) findings. Probably his statement that the excess medium forms a reservoir for yeast growth is also true, but results here recorded give no indication of the mechanism underlying this. Certainly, this reservoir does not serve only to carry through the late larvae, as he suggests.

These experiments show that daily changes of emergence of the first filial generation in 3 × 3 cultures is altered by numerous circumstances which influence yeast growth, adult oviposition, and life-duration and survival of larvae. In a particular type of culture the time-emergence graph is more or less regular in form. In cultures seeded with Hutchi-

son's yeast, for example, it always shows a peak on the second day, followed by a regular decline with usually a secondary rise on the sixth day. That is, the changes in yeast-larval population relationship indicated by previous "Antennaless"-ebony experiments (Sang, 1949*b*) are also found in these normal cultures. In such cultures the relationship between flies, larvae, and yeasts must be relatively stable. But the time-emergence graph can readily be altered by seeding the medium with different kinds of yeast (Hutchison's, Perolin, and brewer's yeast) or by altering yeast growth by adding Nipagin to the medium, and so on. All these changes appear to be produced by *qualitative* changes in the yeasts themselves. Such changes will modify the oviposition rate of the parent-flies (Robertson and Sang, 1944) and so alter the sequence of events which occur in the cultures.

TABLE 7*

SINGLE-PAIR CULTURE: NORMAL MEDIUM WITHOUT NIPAGIN

Day	Yield per Bottle (Flies)	Weight (Mg.)	
		Males	Females
1	12.1	0.935	1.30
2	49.3	0.985	1.26
3	72.6	0.97	1.165
4	61.3	0.85	1.075
5	39.0	0.895	1.105
6	40.1	0.71	0.95
7	48.8	0.78	0.97
8	22.1	0.85	1.015
9	28.6	0.755	0.96
10	28.8	0.72	0.895

* Average values from eight cultures.

4. SINGLE-PAIR MATINGS

To test the conclusion stated above, cultures were prepared with single pairs of newly hatched flies in place of three pairs of 3-day-old flies. The experiments were also designed to show the importance of adult mortality in relation to the course of family growth. Owing to the existence of a pre-oviposition period of 1 day, the use of virgin pairs will allow the yeasts to develop for a longer period before larvae start disturbing the medium. Also, the total number of eggs laid should be considerably less throughout the whole period of culture (Robertson and Sang, 1944).

a) Daily emergence.—Single virgin pairs were placed on normal media without Nipagin and the first count taken 10 days after setting up the cultures. This first-day count is lower than in previous experiments (Table 7). Maximum yield occurs on the third day. Thereafter, there is a fairly regular decline with a marked peak on the seventh day. The result is the same as in previous experiments; but, as we should expect, events take place 1 day later. The form of the time-emergence graph is the same for both sexes, in spite of a slight variation in daily sex ratio. The sex ratio for the 10-day period is 0.95 males : 1 female. The 10-day yield is very high (402), higher than for three-pair cultures under the same conditions.

The weights of emerging flies were recorded daily from samples of twenty flies of each sex. The sum of male and female weights decreases rapidly as the cultures age. There is again a steep rise on the eighth day. Thus not only the number but also the mass of emerging flies changes daily.

b) Adult mortality.—In cultures from which adults are not removed on hatching, the mortality after emergence will influence the form of the time-emergence graph; and removal of flies from bottles within 24 hours of hatching will prevent them from laying eggs. That is, larvae present in the medium after the twelfth day (second day of adult count) will

exist under conditions differing from those found in Pearl's (1926) and Bodenheimer's (1938) work. They will not encounter any competition from second-generation larvae. At the earliest, these larvae will appear as adults on and after the eighteenth day of culture (eighth day of count).

These two points were examined in single-pair cultures without Nipagin. All cultures not containing a pair of living flies on the ninth day were discarded. Ten of the cultures retained were counted daily, and the flies were killed (control). From the remainder, three cultures were chosen at random each day, and the total number of living and dead flies was recorded. In this series (experimental), flies emerging on the following day were also noted. On the last few days all the dead flies were found only with great difficulty, because some had been plowed into the medium by the larvae.

In the control series, emergence reaches its peak on the third day and then falls, with a subsidiary peak on the seventh day (Table 8). The 10-day yield (294) is considerably lower than that of the previous experiment, but general changes are the same. Second-day counts of the experimental series (col. 2) are almost identical with the controls. The 10-day total is 283 and lies well within the limits of experimental error. Hence removal of flies has little influence on larval competition during the later part of the 20-day period. When these two sets are grouped together, the emergence statistics show that variability is highest on the first day, declines rapidly, and becomes constant after the fourth day. Females predominate up to the fourth day, but this has little influence on emergence-time graphs. The 10-day total sex ratio is 0.90 males : 1 female.

Numbers of living flies found in the experimental series (col. 7) increase up to the seventh day and then decline rapidly (Fig. 4). Up to the seventh day the values correspond almost exactly to the total population of the control series. On the seventh day some flies die, and, after this, adult mortality increases rapidly. The total of living and dead flies does not correspond closely with the sum of the control series. The difference is within

TABLE 8

SINGLE-PAIR CULTURE, DAILY AND TOTAL COUNTS

Day	Control (1)	Experimental 2d-Day Count (2)	Mean of Cols. 1 and 2 (3)	σ (4)	Coefficient of Variation (Per Cent) (5)	Total Population (6)	Experimental 1st-Day Count (7)	Deaths (8)	Deaths + Col. 7 (9)
1.....	4.6	2.0	4.0	3.33	82.3	4.0	2.0	.....	2
2.....	35.0	29.7	33.8	15.4	44.3	37.8	46.0	.....	46.0
3.....	53.7	50.7	53.0	10.4	19.6	90.8	102.3	.....	102.3
4.....	40.6	37.0	39.8	6.56	16.5	130.6	109.3	1.7	111.0
5.....	32.8	31.6	32.5	6.29	17.8	163.1	155.0	.....	155.0
6.....	31.5	26.3	30.2	6.68	22.1	193.3	202.0	.....	202.0
7.....	30.2	34.7	31.4	6.98	22.3	214.7	207.3	17.0	224.3
8.....	22.6	22.7	22.6	6.61	29.3	237.3	129.6	82.0	211.6
9.....	18.4	26.7	20.5	6.71	32.7	257.8	96.0	94.0	190.0
10.....	25.5	21.3	24.5	5.79	23.6	282.3	104.0	172.3	276.3
11.....	24.3	26.0	24.8	7.03	28.4	307.1	.....	.....	.....

the range of experimental error and may be due to the difficulty of finding all the dead flies.

Single-pair cultures produce almost as many flies as three-pair cultures. The difference that we should expect from the oviposition experiments is not found. Emergence curves retain their trend but are displaced by 1 day. Changes in the culture as a whole, that is, in the interactions between adults, larvae, and yeasts, can alone account for this. Thus, as found for our oviposition experiments (Robertson and Sang, 1944), we arrive at the conclusion that the course of yeast growth, within certain limits, also determines the course of fly population growth. Adult mortality considerably modifies the emergence curves.

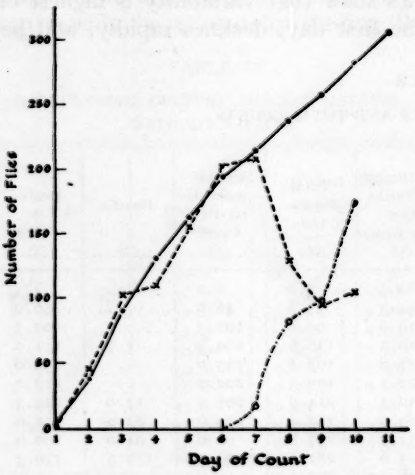

FIG. 4.—Numbers of flies found in single-pair cultures when the flies are not removed on hatching. X = number of living flies found; ○ = number of dead flies; ● = total number of flies emerging.

c) The significance of growing yeast population.—The influence of the growing yeast population can be further examined by cultivating flies on dead yeast media. Such cultures have to be kept sterile throughout the experiment, and this introduces certain technical difficulties. The following procedure was found to be satisfactory. Normal bottles were prepared. After the medium had set, 0.4 gm. Perolin dry yeast was sprinkled over the surface. The bottles were stoppered and autoclaved. After cooling, the yeast was still found on the surface, and the medium was of normal consistency. Flies cultured under sterile conditions (see Gordon and Sang, 1941) were etherized while still in tubes, and virgin pairs were transferred to the cultures with sterile platinum spoons. The bottles were kept on their sides until the flies recovered, thus saving them from sticking to the medium. Any cultures which showed molds or bacterial scum or did not contain a pair of living flies on the ninth day were discarded. When the counts are made, the cultures immediately become infected with yeasts, etc. But the flies which emerge for the next 4 days will be pupae at this time. So contamination will not influence them in any way. By dividing the cultures into random groups, counting the flies emerging in one group for 3 days, discarding these bottles, and going on to the next group and so on, the size of the experiment is kept within reasonable bounds, and the possibility of contamination is removed.

The yield per bottle over the 10-day period (Table 9) is very low (150) on sterile media. The highest yield is not reached until the fifth day, after which there is a slow but steady decline. Therefore, dead yeast modifies both the level and the form of the emergence graph. Perhaps more surprising is the constancy of weight of emerging flies. The level is high (mean ♂ + ♀, 2.14 mg.). This shows that the food supply is adequate throughout. Thus it is probable that there is no prolongation of the larval peri-

od when the adult female lays its eggs on a sterile medium and that changes of emergence frequency are due only to changes of adult oviposition rates. This oviposition rate will be lower and have a different course from that of flies fed on live yeast (Robertson and Sang, 1944). These results confirm those of an earlier experiment by Gordon and Sang (1941).

None of the adults which emerge from these sterile cultures dies before the tenth day of the count. So the high adult mortality found with live yeast (Table 8) must be due to toxic products produced by either the larvae or the yeasts in the medium or to a general deterioration of culture conditions. This result confirms Steinfeld's (1928) finding, namely, that Drosophilae live longest under aseptic conditions. It also serves to emphasize again the important role of the yeasts in the medium.

CONCLUSIONS

From these experiments on the adult population count we see that the sequence of events in a culture is more or less constant. The greatest number of adults emerges on the second or third days, and then numbers decline. On the sixth or seventh day there is usually a greater hatch than on the preceding or succeeding days. From our work on the oviposition of *Drosophila* and on larval survival (Robertson and Sang, 1944; and Sang, 1949*b*) we can conclude that the time-emergence graph is determined primarily by changes of the oviposition rate of the parent-flies and only secondarily by the rate of development and survival of larvae. But oviposition of the parents is determined to some extent by qualitative changes of the yeasts present in the medium; and these changes are brought about by the developing larval population. Thus the course of population growth depends on the interaction between larvae and yeasts, and the phases of this are apparent in the physical changes of the medium. Two of these are reflected in the adult counts of the second to third and sixth to seventh days. They correspond to the spreading of the yeasts into the medium (third day) and the breaking-up of the gel by the larvae (fifth day).

When yeasts of different quality are seeded onto the medium, the time-emergence graph is altered. Again, the adult count is modified when the number of eggs laid by the females is abnormal or when cultures become infected with molds. It is difficult to interpret such changes from the adult counts alone, except in special cases. For example, in sterile cultures the result is probably due to an alteration of the oviposition rate such as we have already shown (Robertson and Sang, 1944). In cultures seeded with Perolin yeast the data suggest that the rate of development of the later larvae may be speeded up. But it is impossible to evaluate the effect of Nipagin, which acts indirectly through the yeasts.

TABLE 9

YIELD ON STERILE MEDIUM

Day	Yield per Bottle	Weight (Mg.)	
		Males	Females
1	1.0	.....	.....
2	6.12	0.95	1.26
3	14.0	0.97	1.13
4	16.0	.....	.....
5	23.9	0.92	1.22
6	20.2	0.94	1.26
7	20.6	0.90	1.32
8	18.5	.....	.....
9	17.1	0.91	1.19
10	12.6	0.84	1.06
11	8.1	0.90	1.21
Total	158.1	.....	.....

These results confirm the importance of qualitative as well as quantitative differences in the yeast population. They also show that the quality of the yeasts changes as the cultures age.

The above conclusions demonstrate the close interdependence of the two populations in the cultures. They also show that the adult count can only indicate changes of parental fertility and of larval life within relatively wide limits. And no dynamic analysis of population growth is possible which does not give due weight to the circumstances modifying preadult life. The oversimplification of the situation implied in the logistic law has led to neglect of this phase of population development. Indeed, Hammond (1939), following Pearl (1926), tends to regard the preadult stages as "embryonic" and therefore unimportant. We have already shown that this view is incorrect (Sang, 1949*b*). It remains for us to point out that a logistic formula cannot be meaningfully applied to the numbers of adults found during the phase of family growth. It can fit the numbers of adults emerging from the cultures only very crudely (see Fig. 2). It cannot fit our numbers for living adults found. Bodenheimer (1938) also finds that adult deaths cause a decline in that population after the seventh day of emergence; thereafter his population goes through a second cycle. This death rate is higher than that found by Pearl, Miner, and Parker (1927) and is due not only to crowding but also to environmental changes produced by the populations in the culture medium, i.e., by the larvae and yeasts.

SUMMARY

1. It is commonly taken that the logistic law adequately describes the numbers of adults emerging from a *Drosophila* culture (Vevers, 1939). This thesis is examined experimentally, as is the hypothesis that the population growth curve is produced primarily by the action of crowding on the fertility and longevity of these adults.

2. It is shown that in this case the logistic has been applied to "family growth" and not to true population growth and that the time-emergence graph for family growth is not always regular in form. In normal cultures there is a peak of emergence on the second to third day and a subsidiary peak on the sixth to seventh day. Such a result cannot be fitted by a logistic curve.

3. Furthermore, emergence is considerably modified by changes of culture conditions; by the oviposition rate of the parent-flies; by the presence of molds, etc., in the cultures; and by the quality or "brand" of the yeast seeded onto the cultures. Within the limits tested, the quantity of yeast seeded has little effect on emergence. But the presence of Nipagin in the medium, by its action on the yeasts, has a decisive effect.

4. The quantity of medium in the cultures also determines their productivity, and Harnly's (1929) findings on this point are confirmed.

5. By itself the adult count tells us little about parental oviposition or about larval survival. These latter are determined to a considerable extent by quantitative and qualitative changes of the yeast population. And these changes are produced, in part, by the action of the developing larvae in the cultures. The feeding larvae alter the environment—the culture medium—and the growth of the yeasts.

6. It is the complex interaction between the two populations in the cultures which determines the daily adult count

by its effects on parental fertility, pre-adult survival, and adult survival. There is no evidence that adult crowding is important in this respect.

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THE ECOLOGICAL DETERMINANTS OF POPULATION GROWTH IN A DROSOPHILA CULTURE

VI. THE TOTAL POPULATION COUNT[1]

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OUR previous studies (Robertson and Sang, 1944; Sang, 1949*a*, *b*) have shown that there is an exceedingly complex interaction between the developing fly and yeast populations present in a normal *Drosophila* culture. And we have emphasized that to understand the dynamics of population growth we must consider changes of all stages of the *Drosophila* population and their relationship to concurrent changes of the yeast population. Bodenheimer's (1938) laboriously performed experiments alone give us some indication of the former; there is no information concerning the latter. The reasons for this lack of data on yeast changes lie in the failure to recognize that the yeasts are the main larval food substance (see Sang, 1949*a*) and in the difficulty of estimating changes of the yeast population, even roughly. It is impossible to separate yeast cells from organic material already present in corn-meal-molasses or banana-agar media. Indeed, the normal *Drosophila* culture cannot be considered the most useful setup

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for studying the interactions between the two species. However, from the preceding work we see it is important that we should obtain some information on modifications of the yeast population and the relation of larval population size to these, as well as the proportions of the various larval stages found at any particular time in the growth of a culture. The work which follows is an attempt at a first approximation to such an analysis.

MATERIALS AND METHOD

Little has been done on yeast growth on solid media, chiefly because of the difficulty of separating yeast cells from the agar medium. The development of a medium (Pearl's S101, modified) suitable for *Drosophila* culture as well as yeast growth and containing only salts, molasses, and agar (Robertson and Sang, 1944) has partially overcome this difficulty. The removal of the various fly stages (eggs, larvae, and pupae) presents a further problem in population experiments. The following techniques were developed for the separation of these two components of the cultures.

To remove the yeasts, bottles containing the medium were boiled for 15 minutes in the autoclave. This liquefied the gel, which was then poured into hot centrifuge tubes and centrifuged for 3 minutes at high speed. The still fluid agar was poured off, and the residue (yeast and agar) was boiled with water for a further 15 minutes. This was then centrifuged as before and the washing repeated twice more. The cake of sedimented yeast was removed from the tubes and dried in an air oven at 90° C. until it attained a constant weight.

Variations in room temperature influence the rate at which the original gel resets. Hence there are variations in the quantity of agar carried down by the yeast when first centrifuged. Further washing does not remove all this agar, for repeated boiling throws it out of solution as a granular precipitate; hence controls without yeast have to be run with each estimation. These controls are always prepared at the same time and from the same batch of media as the experimental bottles.

Because of the viscosity of the fluid agar, all the yeast is not centrifuged down. Attempts to filter off the yeasts and to precipitate the agar by adding salts were unsuccessful. Therefore, the above procedure was adhered to, and a standardization curve was prepared by finding the percentage return when known weights of yeast were placed on the medium. The proportion of yeast recovered after separation was more or less constant for all quantities examined (mean = 76.92 per cent). In the experiments which follow, results will be expressed in terms of quantity of dry yeast recovered.

In population experiments we have to remove eggs, larvae, and pupae as well as the yeasts. This was done as follows: Larvae were made to migrate to the surface of the medium by placing the bottles in a dish of water at 35° C. These larvae were then collected on cool glass rods placed in contact with the medium and suspended from a cork stopper. By slowly raising the bath temperature to 45° C. the majority of the larvae were made to move onto the rods. They were then washed off and stored in tubes of fixative (Kahle's fluid). This collection is best carried out in the dark. Eggs, pupae, and the few remaining larvae were removed with a brush from the centrifuge fluids mentioned above. In this way all stages were collected and stored prior to counting.

EXPERIMENTAL

1. THE GROWTH OF THE YEAST POPULATION

Daily changes of the yeast population on culture media in the absence of flies were examined first. Bottles containing 50 cc. Pearl's S101 (modified) medium were seeded with 2 drops of a 2 per cent suspension of Hutchison's yeast. A control series, without yeast, was also prepared. Both sets of bottles were incubated at 25° C. Each day three experimental bottles were analyzed, and one control bottle was treated to find the agar residue. Detailed results are given in Table 1.

TABLE 1

YEAST GROWTH IN CULTURES WITHOUT FLIES

Age of Culture (Days)	Wt. Yeast + Agar Residue			Wt. Agar Residue	Yeast Weight			Average Yeast Wt. (Gm.)
	1	2	3		1	2	3	
0.....	.....	.....	.....	.....	.....	.....	.....	0.0006
1.....	0.032	0.033	0.043	0.017	0.015	0.016	0.026	0.019
2.....	0.146	0.109	0.096	0.015	0.129	0.092	0.079	0.100
3.....	0.214	0.222	0.203	0.021	0.197	0.205	0.186	0.196
4.....	0.262	0.205	0.287	0.016	0.245	0.188	0.270	0.234
5.....	0.306	0.313	0.317	0.020	0.289	0.296	0.300	0.295
6.....	0.323	0.331	0.307	0.019	0.306	0.314	0.290	0.303
7.....	0.358	0.348	0.352	0.015	0.341	0.331	0.335	0.336
8.....	0.345	0.352	0.324	0.015	0.328	0.335	0.307	0.323
9.....	0.345	0.350	0.363	0.014	0.328	0.333	0.346	0.336
10.....	0.348	0.349	0.360 0.360	Av.=	0.331	0.332	0.343 0.343	0.337
11.....	0.358	0.365	0.342 0.353	0.017	0.341	0.348	0.325 0.336	0.338

Table 1 shows that under our experimental conditions yeast growth takes place in much the same way as, but more slowly than, it does in fluid media (Hartelius, 1934). At first, up to the third or fourth day, growth is rapid. It then declines slowly until the asymptotic population is reached on the tenth day; that is, it follows the usual sigmoid growth curve (Fig. 1). Preliminary observations show that some yeast cells have penetrated to the lower surface of the medium by the fifth day. So the yeasts are growing throughout the medium as well as on the surface, and a gradient of yeast is formed.

The presence of flies in the cultures will alter yeast growth. The role of the larvae in this connection will be discussed below. Here we will note the changes of yeast growth in cultures seeded initially with one and with three pairs of flies. The three experiments were run at different times. As before, each result is the mean of three cultures (Table 2 and Fig. 1).

The presence of flies in cultures decreases the quantity of yeast recovered. This is apparent on the first day, but the difference found may not be significant. Three pairs of flies lower the quantity of yeast found, more strikingly than does one pair (Fig. 1). But one pair produces a proportionally greater lowering of the asymptotic yeast population. Maximum yeast quantity is reached on the sixth (1 pair) and fourth (3 pair) days instead of the tenth day (without flies). Thereafter the yeast population declines slowly in

both fly-containing cultures. This decline is irregular and may be caused by a changing equilibrium between larval and yeast populations. The occurrence of molds (which raises our estimate for yeast quantity) in cultures after the seventh to eighth day makes the later estimates increasingly inaccurate. Hence the fluctuations may be a secondary phenomenon.

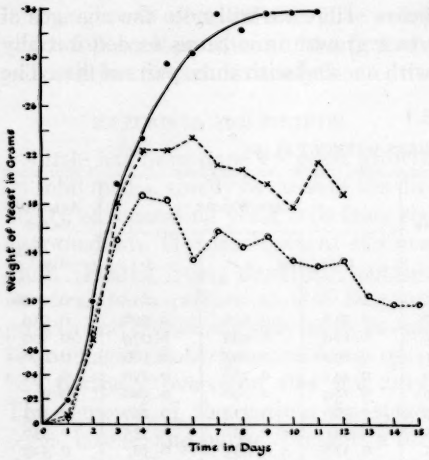

FIG. 1.—Growth of the yeast population in *Drosophila* cultures. ●, yeast growth in cultures without flies; *X*, growth in single-pair cultures; ○, growth in three-pair cultures.

2. THE MASS OF FLY STAGES IN THE CULTURE

The issue raised in the last paragraph, namely, the relationship between larval population and yeast growth, demands an estimation of the *mass* as well as of *numbers* of the different fly stages present in growing cultures. This has been neglected in Bodenheimer's (1938) estimates of population development. Such an estimate is important not only in relation to yeast growth but also as a measure of the increase of living material from day to day.

The eggs, larvae, pupae, and flies separated from the cultures were counted and classified into stages (Table 5). The larvae, etc., from the three cultures analyzed on each day were then placed together and dried at 90° C. until they reached a constant weight. A third of this weight is taken as representing the average mass of the stage on any day. Certain errors are introduced into this estimate and into the counts. Eggs which do not hatch are counted on successive days until they turn brown and can thus be recognized as inviable and then be discarded. Dead larvae and pupae are also included in the counts, for they, too, cannot be recognized. These errors are of a minor order and will be neglected in discussing the results. The mass of eggs found on any day was too small to be measured accurately, and it has also been neglected.

TABLE 2

YEAST GROWTH IN *Drosophila* CULTURES

(In Grams)

DAY	NO. OF FLIES		
	None	1 Pair	3 Pairs
1	0.019	0.007	0.005
2	0.100	0.069	0.073
3	0.196	0.181	0.150
4	0.234	0.224	0.187
5	0.295	0.224	0.183
6	0.303	0.234	0.134
7	0.336	0.211	0.158
8	0.323	0.208	0.145
9	0.336	0.196	0.153
10	0.337	0.177	0.134
11	0.338	0.215	0.130
12	.....	0.188	0.133
13	.....	.....	0.105
14	.....	.....	0.098
15	.....	.....	0.098

The larval mass increases rapidly in both sets of cultures until it reaches a maximum on the fifth day (Table 3 and Fig. 2). As we should expect, this maxi-

mum is considerably higher in the three-pair cultures. After the fifth day the larval mass declines and, in the three-pair cultures, falls to a more or less constant level. The first pupae appear on the fifth day, and the pupal mass increases until the eighth day. In single-pair cultures the pupal mass then decreases rapidly, while in the three-pair cultures approximately the same maximum mass is found for a further 2 days before there is a rapid decline. Probably an increase of the duration of preadult life in the three-pair cultures produces this spread. The adult mass begins to increase on the ninth day in both sets of cultures.

The mass of living material, the *Drosophila* biomass, increases rapidly at first in both sets of cultures. It reaches a maximum on the eighth and seventh days in the one- and three-pair cultures, respectively. In the former a rapid decline follows this; in the latter the decline is at first less pronounced. This falling-off of weight is brought about by (*a*) a decrease in the larval mass; (*b*) at the same time, a real decrease instead of an increase in the pupal mass; and (*c*) after the ninth day, a further decrease of the pupal mass which is not compensated for by the increase in adult mass. Unfortunately, it is impossible to relate changes of biomass to the addition that we should expect to occur when one stage transforms into another. The data are inadequate for such a calculation. Clearly, adults will weigh considerably less than the pupae from which they emerge, since they leave their pupa cases behind. These form approximately 40 per cent of the dry weight of

TABLE 3

CHANGES OF THE FLY BIOMASS IN *Drosophila* CULTURES (IN GRAMS)

Fly Mass	Day													
	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Single pair:														
Larval mass	0.0003	0.0019	0.0080	0.0251	0.0221	0.0187	0.0126	0.0094	0.0094	0.0043				
Pupal mass	.....	.....	.....	0.0033	0.0241	0.0396	0.0740	0.0682	0.0552	0.0286				
Adult mass	.....	.....	.....	.....	.....	.....	.....	.....	0.0043	0.0195				
Total	0.0003	0.0019	0.0080	0.0284	0.0462	0.0583	0.0866	0.0776	0.0689	0.0514				
Three pairs:														
Larval mass	0.0004	0.0042	0.0235	0.0395	0.0312	0.0268	0.0093	0.0096	0.0096	0.0067	0.0085	0.0049	0.0056	0.0034
Pupal mass	.....	.....	.....	0.0081	0.0570	0.0665	0.0671	0.0734	0.0421	0.0218	0.0139	0.0161	0.0174	0.0196
Adult mass	.....	.....	.....	.....	.....	.....	.....	0.0045	0.0257	0.0346	0.0307	0.0374	0.0319	.....
Total	0.0004	0.0042	0.0235	0.0476	0.0882	0.0933	0.0764	0.0875	0.0774	0.0631	0.0531	0.0584	0.0549	.....

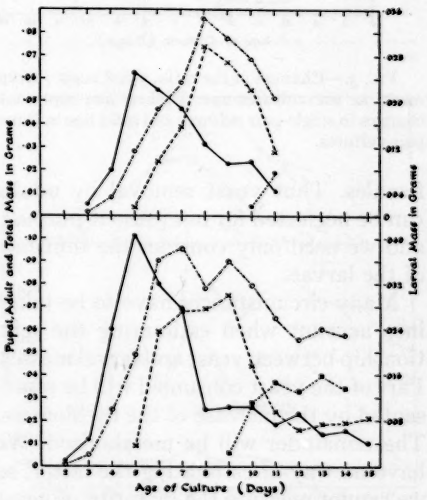

FIG. 2.—Daily changes of the *Drosophila* biomass in single-pair (*upper figure*) and three-pair cultures (*lower figure*). ●, larval mass; *X*, pupal mass; □, adult mass; ○, total biomass. Weight of larvae is represented on a different scale from the others.

the pupa. In our cultures a real decrease of the number of pupae as well as of larvae partly accounts for the fall in total weight after the eighth day, and this is undoubtedly an experimental error.

3. LARVAL MASS AND YEAST GROWTH

Two stages of the fly feed on the yeasts, the adult and the larva. Preliminary experiments show that three pairs of adults consume less than 0.001 gm. (dry weight) of yeast per day. The major part of this quantity is consumed by the

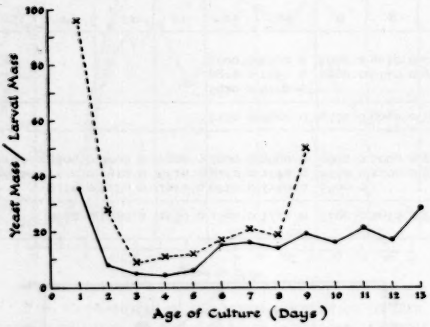

FIG. 3.—Changes of the ratio, yeast mass : larval mass, as the cultures age. Broken line represents changes in single-pair cultures and solid line in three-pair cultures.

females. Thus yeast removal by adults can be neglected for our present purpose, and we need only consider the influence of the larvae.

Many circumstances have to be taken into account when estimating the relationship between yeast and larval masses. Part of the yeast consumed will be represented by the increase of the fly biomass. The remainder will be metabolized. We have no way of estimating the latter, so we cannot measure the quantity of yeast which has developed in the cultures. The data suggest, however, that the larvae may at first encourage yeast growth. Similarly, we cannot measure the availability of yeasts to the larvae. Since larvae feed for the most part either on the surface or with their bodies penetrating vertically into the medium, the amount of available yeast will depend on the quantities present at these two locations. These quantities will be a complex function of the history of the culture.

In this situation, which depends on variables such as the rate of diffusion of yeast food substances through the medium and size of larval mouth parts, we can, therefore, construct only a coarse index to describe the interaction between the two populations. It is the ratio of yeast mass to larval mass. If we bear the above qualifications in mind, we can consider it as a rough estimate of larval feeding opportunity.

Figure 3 shows that on any day the quantity of yeast per unit of larval mass is greater in one- than in three-pair cultures. In both cases feeding opportunity decreases rapidly as the larval populations increase. It reaches a minimum on the fifth (one-pair) and fourth (three-pair) days. Thereafter there is a slight increase, which reaches a higher level for the one-pair cultures. These changes are sufficient to account for the differences of weight of the flies emerging from the cultures (Table 4). Flies hatching during the first 3 days of count will have relatively more favorable larval conditions, as will those hatching after the eighth to ninth days. But the correspondence between feeding opportunity and adult size will not be high because of the change in duration of the preadult period. This increase in feeding opportunity on the eighth to ninth days cannot account for the late rise of adult weight, for we have seen that this occurs earlier when the larvae are more crowded (Sang, 1949*b*). It must be due to a qualitative change of the yeasts.

The total mass of living material produced in culture bottles (fly + yeast masses) is decreased by having flies in the cultures. The three-pair culture produces less than the one-pair culture. This decrease, when compared with the yield in cultures without flies, is roughly proportional to the fly biomass. But we cannot be certain that this quantity represents yeasts metabolized by the flies. Nor can we say whether larvae stimulate or inhibit yeast growth. The yeasts stop growing only after the larval population has started to decline.

4. THE FLY POPULATION

The numbers of eggs, larvae, pupae, and adults found as cultures develop were recorded. The larvae were further classed into three size groups. These larval groups were chosen on the basis of the instar sizes given by Alpatov (1929). According to his measurements, larvae up to 1.50 mm. are in the first instar, from 1.60 to 2.9 mm. in the second instar, and from 3.1 to 4.8 mm. in the third instar. But these limits may be incorrect when larvae are starved; certainly, the difference between one stage and the next becomes less distinct as cultures age. It was hoped that a mathematical examination of these data would give some information on the growth and transformation of the different stages. So far this expectation has not been fulfilled, and we shall content ourselves by describing, as Bodenheimer (1938) does, the numerical changes of the population.

The numbers of all stages taken together increase rapidly and then decline (Fig. 4). The three-pair cultures reach this maximum (6 days) more rapidly than do the one-pair cultures (8 days), and the increase of numbers is more irregular in the former. This earlier attainment of a maximum is due to the relatively greater number of larvae produced. The three-pair cultures contain almost twice as many individuals as the maximum (Table 5). Bodenheimer's (1938) cultures do not reach their first peak until the eighth day; then the total population is approximately the same as in our three-pair cultures. Hence the two experimental procedures give different results and cannot be compared in detail. The decline of the population which

TABLE 4

WEIGHT OF FLIES EMERGING FROM SINGLE-PAIR CULTURES

Day	Mean ♂+♀ Weight (Mg.)	No. Emerging
1	1.04	39
2	1.10	34.5
3	1.08	28.5
4	0.85	23.6
5	0.82	17
6	0.77	10.1
7	0.66	7
8	0.71	6.4
9	0.69	5.1
10	0.63	2.2

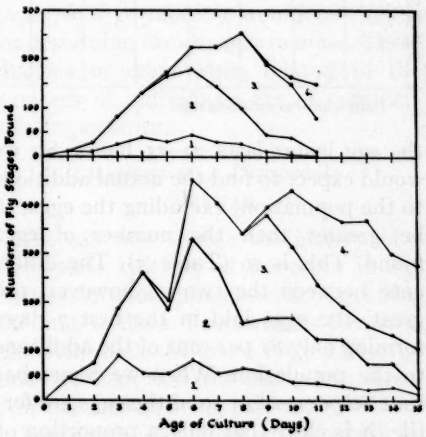

FIG. 4.—The total fly population in single-pair (*upper figure*) and three-pair (*lower figure*) cultures. *1*, number of eggs; *2*, number of larvae; *3*, number of pupae; *4*, number of adults.

occurs after the maximum is reached is due, in both Bodenheimer's and our own experiments, to an increase of larval mortality. In our experiments there is also a fall in the number of pupae which is not compensated for by the emerging adults. This error is due to sampling.

The number of eggs found and counted every 24 hours will be slightly less than the number actually laid, for the egg instar lasts 22–23 hours. So we would expect to find the actual additions to the population, excluding the eggs, to be greater than the number of eggs found. This is so (Table 5). The difference between the two is, however, too great, the eggs laid in the first 7 days forming only 81 per cent of the additions to the population. When we remember that 80 per cent or so of the eggs are fertile, it is clear that only a proportion of the eggs laid are actually recovered (Robertson and Sang, 1944). Assuming that this proportion remains constant, we find that the number of eggs laid in both cultures is very small and that the maximum oviposition is reached much later than when flies are supplied with fresh food daily. This confirms our previous conclusion (Robertson and Sang, 1944).

The larval stage lasts for about 4 days. In single-pair cultures the number of first-instar larvae form about 80 per cent of the daily additions to the population during the first 5 days. This means that the stage lasts 19.2 hours, which is in close agreement with Alpatov's (1929) mean value of 20 hours. From the fifth day onward, this percentage increases. That is, the rate of larval development is slowed down. This is in agreement with what we should expect from changes in available yeast. Similar changes occur for the second and third larval instars, but they are not so readily interpreted. For example, the number added to the third-instar group will depend on the condi-

TABLE 5

NUMBERS OF FLY STAGES RECOVERED

Day	One-Pair Cultures							Daily Additions*	Three-Pair Cultures						
	Eggs	Larvae				Pupae	Adults		Eggs	Larvae				Pupae	Adults
		I	II	III	Total					I	II	III	Total		
1	10							.....	46						
2	10	13	4	.....	17			17	36	38	.....	.....	38		
3	23	32	18	6	56			39	89	40	51	7	98		
4	40	28	34	27	89			33	56	69	61	73	203		
5	30	43	38	55	137	6		54	54	39	41	53	133	18	
6	43	47	45	42	134	47		38	82	49	84	114	247	124	
7	30	27	46	42	115	81		18	53	44	63	98	206	148	
8	27	21	27	25	73	156		30	66	14	23	56	93	180	
9	13	7	17	20	44	144	1	.....	57	23	29	60	112	213	15
10	6	4	12	16	32	114	15	.....	37	23	47	61	131	148	93
11	3	0	5	4	9	68	71	.....	37	10	28	52	90	108	157
12									32	6	24	59	89	71	175
13									23	4	11	36	51	79	217
14									28	2	5	41	48	60	167
15									14	1	1	12	14	79	171

* Daily additions excluding eggs.

tions to which the transforming larvae have been submitted in the two previous instars. In the same way and for the same reasons, it is difficult to analyze the changes of numbers of pupae. This stage lasts about 4 days. The number of pupae formed from the fifth to the eighth day is slightly greater than the additions to the larval population during the corresponding period (second to fifth day) in the one-pair cultures. This suggests that there is little or no mortality in the larval stage up to the fifth day. After the eighth day the changes become more complex, and the data are inadequate for the reasons given above.

In three-pair cultures the changes are more complex. This is due to the greater number of larvae present in the medium, which brings about an earlier prolongation of the larval period, as is indicated by the fall in pupal weight discussed below. In addition, the graphs suggest that here there is an earlier onset of larval mortality. In this set, if we compare the number of flies emerging over a 10-day period with the number of eggs laid in the first 10 days, we find that only 39 per cent of the eggs survive to the adult stage. This is not an accurate comparison, for we know that many of the flies will take more than 10 days before they emerge. Taking an outside limit of 15 days for the slowest larvae, we find that, even then, only 44 per cent survive. Eighty per cent of the eggs laid in single-pair cultures survive. But this number represents only 69 per cent of the new members added to the population when we allow for the fact that only a proportion of the eggs is actually recovered.

When we compare the numbers with the mass of *Drosophila* found (Fig. 2), we find that the rate of increase in the total population is slower in the latter than in the former. In three-pair cultures the maximum mass is not reached until later than the maximum number, and in both sets of cultures the mass increase is more regular. These differences are due to the fact that the initial increase of larval numbers is greater than the corresponding increase of larval mass. In three-pair cultures the decline in larval mass occurs more rapidly than the fall in numbers. In single-pair cultures mass and numbers of pupae correspond almost exactly. That is, the pupae formed on successive days are of almost the same weight. In the three-pair series there is no such correspondence, and after the seventh day pupal weight declines; hence smaller pupae must be formed. In the same way the number and mass of adults do not correspond, for the emerging adults also have successively smaller weights.

While it is impossible to relate number and mass exactly, since pupae, for example, will decrease in weight as they age, it is clear that a description of the numerical changes of a population is itself inadequate. The formulae applied by Bodenheimer (1938) to numbers in a *Drosophila* population would be useless for describing the changes in mass. These changes in mass when related to the numbers of individuals are of considerable importance.

CHANGES IN A *Drosophila* CULTURE

We can now divide the history of a *Drosophila* culture, as it occurs in our bottles, into four phases occurring during the first 10 days. To make this comparable with Bodenheimer's (1938) description, we shall take the changes in three-pair cultures as being typical.

0–2 days.—Numbers of eggs laid increase and depend on the number of adults seeded; the larval mass and numbers increase rapidly. The quantity of

yeast available to these is considerable, and the total yeast quantity is increasing.

2–5 days.—The egg population rises to its maximum. The larval mass increases further and reaches its maximum. The yeast reaches its asymptotic level, and larval feeding opportunity declines. Consequent on this, the larval stages become prolonged.

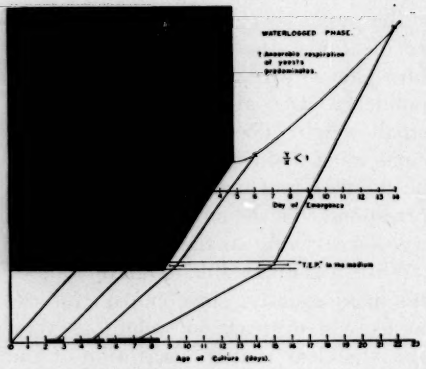

FIG. 5.—Schematic representation of events underlying the changes in exhibition of the gene "Antennaless." The exhibition-time graph is shown at the top. The bottom horizontal line shows the spread of oviposition corresponding to a particular day of emergence; and the middle axis, which is arbitrarily placed, shows the spread of the effective period of gene action. During the first phase (aerated phase) gene exhibition falls; in the second phase it recovers again. Qualitative changes of the yeast account for this cycle.

5–8 days.—Egg population declines. Both larval numbers and mass also decline, and larval mortality may become considerable. Pupae appear, and numbers and mass increase rapidly. Total yeast decreases, but larval feeding opportunity is more or less constant.

8–10 days.—Egg population declines still further, and, while larval numbers maintain themselves, the larval mass decreases. The number and mass of pupae also begin to decline, and the first adults appear. While total yeast decreases still further, feeding opportunity increases slightly.

This preliminary description of the history of the fly and yeast populations is necessarily incomplete and inadequate. Changes of the yeast population are *qualitative* as well as quantitative. We have previously suggested that qualitative yeast changes do occur and that they influence larval development (Sang, 1949*b*). Gordon and Sang (1941), using the mutant "Antennaless" as a biological indicator of yeast quality, have shown that this is so and that the change is a continuous one in normal cultures. The activity of the larvae in the medium changes the yeasts as the cultures develop. This change passes through two phases: up to the fifth day the yeasts contain a greater proportion of substance or substances (*Y*) which inhibit the exhibition of the gene "Antennaless"; after the fifth day they contain a greater proportion of substance or substances (*X*) which encourage gene exhibition (see legend, Fig. 5). At least one of these substances is a vitamin (riboflavin), and vitamins have been shown to influence the rate of development, size, and survival of *Drosophila* larvae (Schader, 1940; Tatum, 1941). Thus changes of the vitamin content of the yeasts could produce the observed results.

The *Drosophila*-yeast system is not stable. The balance between the two populations that we might expect on theoretical grounds (Gause, 1934; Kostitzin, 1937) is not attained in ordinary cultures. The quantity of medium, which forms the food supply of the yeasts, is almost exhausted before the second generation of flies has developed. The surface of the medium has deteriorated, and the yeast cells are "lost" below the level to which the larvae can burrow. After this

stage is reached, the population must decline (see Bodenheimer, 1938). The addition of fresh medium is difficult except by special methods (L'Héritier and Tessier, 1934), and we have no measure of the quantity required to maintain normal population growth. In these circumstances it can be only by chance that Pearl (1926) added a quantity which permitted his fly population to grow according to the logistic law. Observation also shows that the yeasts are rapidly removed from the feeding range of the larvae in crowded cultures. They continue to grow below this level, so that a reserve is formed. In cornmeal and similar cultures, the corn particles may form a further "refuge" for yeast growth in a way similar to that described by Gause (1934). If this is so, the relationships described here may not be true of cornmeal or banana-agar cultures.

Bodenheimer (1938) calculates two indices from his data; one represents changes in environmental resistance, the other the intensity of the struggle for life during the growth of a population. Such indices may be important if we are considering intensity of selection or interspecific competition as it occurs in experimental populations. The first index, environmental resistance, is calculated as 1 *minus* the degree of realization of potential increase. This latter is calculated from the ratio of eggs laid to potential oviposition rate, which is taken as constant from day to day. Our results show that this assumption is incorrect; for oviposition rate, even under the most favorable conditions, changes from day to day (Robertson and Sang, 1944). Clearly, *this index is applicable only to adults*. The intensity of the struggle for existence is the ratio of potential increase of population growth rate to actual growth rate of the total population. The growth rate of the population is estimated as the total population divided by the total population of the previous day. In this case the index is a measure of the struggle *involving the entire population.*

All changes of the population are summarized by the two indices, so we need consider only their daily fluctuations. From his figures, Bodenheimer concludes that (*a*) potential increase is proportional to the number of adult flies present in the culture; (*b*) environmental resistance is negative between the second and seventh days and only increases thereafter; and (*c*) the intensity of the struggle for existence "is very low as long as only the adults of the parental generation exist. With the beginning of oviposition in the filial generation, the struggle for existence grows rapidly and follows in its intensity an exponential curve until the first maximum is reached on the 15th to 17th day. Later it passes to its absolute maximum, when the heavy drop in total population begins; as a rule, it grows smaller steadily afterwards." None of these statements falls into line with our results.

Two major errors of interpretation are clear. The first lies in the confusion over adult and total populations, italicized above. If we assume optimal conditions for egg and pupal survival, then environmental resistance and intensity of the struggle for existence will affect only the two feeding stages—the adult and the larva. These live in different contiguous environments, which, while they interact with each other, are none the less distinct. So both feeding stages have their separate "environmental resistance" and "struggle for life." The second error arises from neglect of yeast population changes. It is difficult to see how any estimate of the realization of potential increase and, therefore, of environmental

resistance can be made without taking these into account. Related to this is the fact that larvae of different stages or age groups will live under divers environmental conditions. For example, during the fourth day of culture the newly hatched larvae will find an abundance of yeast available near the medium surface, while, at the same time, 3-day-old larvae may find a scarcity in the depth at which they are feeding. That is, at any one time each larval batch will have a different "struggle for life." These errors of interpretation make the indices of very dubious validity.

Our figure of yeast available per unit larval mass (Fig. 3) might be taken as an index of the larval struggle for life; but it neglects qualitative changes of the yeast population as well as the many other circumstances already enumerated. The weight of emerging flies might also be chosen as an index, but we have seen that flies hatching on the later days of emergence arise from larvae which have spent very different times in the medium; and the length of larval period is certainly related to the struggle for life. Similarly, the mortality of any larval batch is not simply determined in relation to environmental changes. Indeed, the complexity of ecological changes in a *Drosophila* culture is so great that it will be impossible to construct any simple indices until more is known of the mass physiology of the fly.

SUMMARY

1. A method is developed for the quantitative separation of both fly and yeast populations from the medium in the *Drosophila* culture bottle. The daily changes of these two populations are examined.

2. When there are no flies in the cultures, the yeast population has a sigmoid growth curve. When one or three pairs of flies are seeded, the yeast recovered is decreased, and the greatest quantity is found on the sixth (one pair) or fourth (three pairs) instead of the tenth day. Thereafter, the yeast declines in the fly-containing cultures.

3. The numbers and mass of the various fly stages (eggs, larvae, pupae, and adults) also change as the cultures age. This change is complex, for the rate of development, size, and survival of larvae and pupae are modified by the changing conditions of the cultures. One of these—the yeast available to the larvae—is estimated, and it is shown that the ratio of yeast to larval mass declines to a more or less constant level after the second to third day.

4. A description is also given of four phases occurring during the first 10 days in the development of a culture, and the significance of qualitative changes of the yeast population are also discussed. The relevance of Bodenheimer's (1938) indices of survival in a *Drosophila* culture are examined in the light of these results.

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BREEDING PERFORMANCE OF RATS EXPOSED REPEATEDLY TO 18,000 FEET SIMULATED ALTITUDE[1, 2]

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IN A review Monge (1943) pointed out that the exposure of man and animals to high altitude adversely affects their reproductive potential. This matter is of economic importance to dwellers of the high plains of the Andes, and it is also a matter of concern to individuals who ascend to high altitudes for relatively short intervals as explorers or as aviators.

In earlier studies there had been no agreement as to the nature and extent of the injury induced by exposure to various high altitudes from 11,000 to 27,000 feet. Alders (1930) found that altitudes as high as 25,000 feet did not affect the female sex organs. Nelson and Burrill (1944*b*) found that estrous cycles were unaffected at 18,000 feet simulated altitude. In a large-scale expedition to Mount Evans (14,260 ft.) Moore and Price (1948) found that the breeding performance of imported rats was unaffected during a 2-month period. Other investigators (Loewy and Heller, 1933; Sundstroem and Michaels, 1942; Monge, 1943; Monge and San Martín, 1945; Monge, San Martín, Atkins, and Castañón, 1945; Altland, 1949) have reported reduced fertility, faulty gestation, abortion, death during pregnancy and parturition, and genital tract lesions at various high altitudes. Much of the conflicting data is due to differences in method and degree of exposure. There is a need for further investigation on the nature of the response of such critical reproductive functions as breeding, gestation, parturition, and postnatal development to an altitude exposure sufficiently high to bring about a significant acclimatization response without altering growth and development. In preliminary studies it was found that 4-hour daily exposures to a simulated altitude of 18,000 feet provided the necessary conditions for such an experiment.

[1] A preliminary report was presented to the American Society of Zoölogists (*Anat. Rec.*, 1947).

[2] Gratitude is expressed to Mr. Samuel M. Poiley, animal husbandryman at National Institutes of Health, for valuable data on breeding behavior of control rats of Sprague Dawley colony. Acknowledgment is also due Mr. John Simpson for technical assistance.

METHODS

Male and female rats (Sprague Dawley) were exposed to a simulated altitude of 18,000 feet (b.p., 379 mm. Hg; pO_2, 79.6 mm. Hg) in the same manner as previously described (Altland, 1949). Ventilation in the altitude chamber afforded complete change of air every 2 minutes, and a temperature between 23° and 25° C. was maintained.

The rats were divided into two age groups: in the first group (ser. 1) the exposures were begun at 14 days of age and continued daily through 1 year; in the second group (ser. 2) the exposures were begun when the rats were 125 days old and were continued for 250 days. In breeding experiments the rats of series 1 were housed with 2 fertile controls after 80 exposures; those in series 2 were housed with 2 fertile controls after the first day of exposure.

Pregnancies were determined by observation of daily body-weight changes and by vaginal smears. During the course of preliminary experiments, the exposed females produced fewer offspring than did the controls. In order to eliminate the complicating factor of unobserved cannibalism and to uncover intrauterine abnormalities, laparotomies were performed upon exposed and control rats. In all cases the first operation was performed during the second pregnancy. Light ether anesthesia was employed, and the operations were completed within 15 minutes. A mid-ventral incision of sufficient size was used to permit observation with a minimum of manipulation. The rats were not exposed for 2 days following the operation, and in cases of slow recovery a longer rest interval was provided.

The fetal sites from autopsy material were fixed in 10 per cent neutral formol or in Bouin's fluid. Frozen sections were stained in oil red O, and paraffin sections were stained with eosin methylene blue, Van Gieson's stain, and Berlin blue method for demonstrating iron. Blood analyses were made from tail blood samples. Hematocrit values were determined by use of Van Allen tubes with centrifugation at the rate of 2000 r.p.m. for 30 minutes. Oxyhemoglobin concentrations were determined with the aid of Beckman spectrophotometer, using the extinction coefficients reported by Horecker (1943).

OBSERVATIONS

EFFECT UPON THE MALE REPRODUCTIVE SYSTEM

From calculation of organ/body-weight ratios and from a histological analysis, it was found that the testis and epididymis were unaltered by exposure to 18,000 feet altitude. The seminal vesicle/body-weight ratios were below control values (mean, 35 per cent). Care was taken to avoid loss of seminal fluid during dissection, and all seminal vesicle weights included only those glands which were full of fluid. There was no histologic alteration in the seminal vesicle epithelium. An examination of wet films of spermatozoa smears taken from the epididymis of 20 rats exposed over 200 days revealed normal motility in all cases.

The reproductive capacity of the exposed males is given in Table 1. It was found that there was no significant reduction in the number of males of series 1 that were able to mate and produce 1 litter when mated with fertile controls, but there was a significant reduction in the number of these exposed males that were able to reproduce successfully at regular intervals during the experiment. In series 2 it was found that there was a significant reduction in the number of rats that could produce even 1 litter

TABLE 1

NUMBER OF RATS PRODUCING OFFSPRING WHILE BEING EXPOSED TO 18,000 FEET SIMULATED ALTITUDE, 4 HOURS DAILY

		No. Bred	No. Fertilized	Per Cent Fertilized	Chi-Square Value	No. Producing 3 or More Litters	Per Cent of Total	Chi-Square Value
Exposed Series 1	male	27	18	66.6	3.1	14	51.8	6.57*
	female	59	37	62.7	6.8†	24	40.6	29.5†
Exposed Series 2	male	18	9	60.0	6.1*	2	11.1	28.3†
	female	27	26	96.2	.....	13	48.1	7.4†
Control	male	150	127	85.0	.....	127	85.0	.....
	female	516	430	84.0	.....	430	84.0	.....

* Values above 5.4 show significant reduction in fertility at 0.02 level.
† Values above 6.6 show significant reduction in fertility at 0.01 level.

TABLE 2

LITTER SIZE AND SURVIVAL OF YOUNG OF RATS EXPOSED TO 18,000 FEET SIMULATED ALTITUDE, 4 HOURS DAILY

Litter Size	Control*				Series 1†				Series 2†			
	No. Litters	Per Cent	Total Young at Birth	No. Survived to 21 Days	No. Litters	Per Cent	Total Young at Birth	No. Survived to 21 Days	No. Litters	Per Cent	Total Young at Birth	No. Survived to 21 Days
1	1	0.8	1	1	5	10.0	5	2	5	10.0	5	2
2	.....	.....	.....	.....	7	14.8	14	7	2	4.2	4	2
3	7	5.7	21	20	7	14.8	21	11	9	19.1	27	12
4	8	6.5	32	30	11	23.4	44	32	6	12.7	24	18
5	9	7.3	45	45	6	12.7	30	23	10	21.2	50	20
6	22	17.9	132	103	3	6.3	18	16	1	2.1	6	5
7	15	12.2	105	92	4	8.5	28	21	4	8.5	28	16
8	17	13.8	136	132	2	4.3	16	12	7	14.8	56	35
9	15	12.2	135	123	2	4.3	18	6	2	4.3	18	17
10	13	10.6	130	128	.....	.....	.....	.....	1	2.1	10	10
11	7	5.7	77	65	.....	.....	.....	.....	.....	.....	.....	.....
12	3	2.4	36	33	.....	.....	.....	.....	.....	.....	.....	.....
13	6	4.9	78	68	.....	.....	.....	.....	.....	.....	.....	.....
Total	123	.....	928	840	47	.....	194	130	47	.....	228	137
Survival in per cent	.....	.....	.....	90.5	.....	.....	.....	67.0	.....	.....	.....	60.0
Average litter size	.....	.....	7.6	.....	.....	.....	4.1	.....	.....	.....	4.8	.....

* In 1,529 litters, 13,201 young were born, 11,844 survived to 21 days (89.7 per cent).
† Series 1 exposed since 14 days of age; series 2 exposed since 125 days of age.

and that there was a very great reduction in fertility as the exposures continued. Only 4 males in series 1 and 1 rat in series 2 showed any evidence of normal breeding performance; yet all young that were produced by the exposed males in both series were normal in all respects.

indicated a marked reduction in fertility (Table 1). It was observed that the number of young in the litters which were born to these exposed rats appeared to be smaller than in controls. As indicated in Table 2, the average litter size for exposed rats was as low as 4.1 young per

TABLE 3

NUMBER OF FETAL SITES IN PREGNANT RATS, INCLUDING FREQUENCY OF OCCURRENCE OF FETAL RESORPTION*

Total No. Fetal Sites	Controls Killed and by Laparotomy				Exposed Series 1 by Laparotomy†								Exposed Series 2 by Laparotomy‡				
	No. Preg. without Resorp.	No. Preg. with Resorption			No. Preg. without Resorp.	No. Preg. with Resorption							No. Preg. without Resorp.	No. Preg. with Resorption			
		No. Resorp. Sites				No. Resorption Sites								No. Resorption Sites			
		1	2	3		1	2	3	4	5	6	7		1	2	3	5
1						2											
2													1				
3	1	1			5									1			
4					2		2		1				2	1		1	
5	1						1		1				4				
6	1		1		2			1	1						1		
7	6			1	6	2		1	2		1		3		2		1
8	21	5			6	2	3	2		1	1		3	1	1	1	2
9	32	5			3	1					1				2		
10	14	1	2		2		1	1	1		1		3		1	1	
11	11	4	2	2								2					
12	8	3	1	1													
13	1	2															
14	1	1															
15	1	2															
Total	98	24	6	6	26	7	7	5	6	1	4	2	16	3	6	3	3
Preg. with resorp.	26.9 per cent				55.1 per cent								48.5 per cent				

* Operation or autopsy performed during last third of pregnancy.
† Exposed 4 hours daily since 14 days of age (18,000 feet simulated altitude).
‡ Exposed 4 hours daily since 125 days of age (18,000 feet simulated altitude).

EFFECT UPON FEMALE REPRODUCTIVE SYSTEM

The exposures had no effect upon the occurrence of puberty, on cyclic estrous, or on growth. In preliminary experiments the failure of a large number of the exposed rats to breed at regular intervals

rat as compared with 7.6 young per litter in controls. In the second experiment, when exploratory laparotomies were performed to permit an actual count of the number of fetal sites, it was found that the counts made at birth were not an accurate index of the number delivered.

The results of the laparotomy studies are given in Table 3. It was found that the mean number of implantation sites per uterus in the exposed rats of series 1, including fetal resorptions, was found to be 7.0 sites per uterus. When fetal resorptions were excluded, the mean number was reduced to 5.3 living young per uterus. A similar reduction was found in the exposed rats of series 2. In the controls the mean number of implantation sites per uterus was 9.5, a value which was reduced to 9.1 after considering resorptions. From such calculations it was possible to demonstrate that there was a significant reduction in the number of implantation sites per uterus in the exposed rats, that fetal resorption was a greater factor in reducing litter size in exposed rats than in controls, and that cannibalism or some other factor played a part in the reduction of the litter size. The last statement was possible from the evidence that the observed number of 5.3 living fetuses per uterus is replaced by an actual count of 4.1 young per litter after birth (Table 2).

Additional evidence for there being a reduction in the number of implantation sites in exposed rats was found in the various instances of small litters being observed *in utero* (Fig. 1). There seems to be no doubt that some of the litters of 4 or 5 young, observed soon after birth in the preliminary experiments, actually represented the full complement of young for the litter.

Fetal resorption was found in the exposed rats of both series. Fetal resorption was also found in controls, but it may be noted in Table 3 that, of the 36 control litters that possessed resorption sites, 24 had only one resorption per uterus and the other 12 had two or three sites per uterus. When fetal resorption was discovered in exposed rats, there was considerable variation in the number of fetal sites undergoing resorption (Figs. 2 and 3). As many as seven resorbing gestation sacs have been found in one rat, but more commonly two, three, or four were found. There was variability in the size of the resorption sites, which indicated that there was no uniform time at which all the fetuses died. In this respect the resorption due to hypoxia resembles that found occurring spontaneously in the Albany strain of rats (Burack *et al.*, 1939).

In series 1 the increase in the resorption number was not significant until after 250 days of age (Table 4). Eighty-seven per cent of all exposed rats above

TABLE 4

OCCURRENCE OF FETAL RESORPTION IN RATS OF DIFFERENT AGES EXPOSED DAILY TO 18,000 FEET SIMULATED ALTITUDE

	1–200 Days				201–50 Days				251–300 Days				301–75 Days			
	No. Litters	No. Fetal Sites	No. Resorptions	No. Litters with Resorption	No. Litters	No. Fetal Sites	No. Resorptions	No. Litters with Resorption	No. Litters	No. Fetal Sites	No. Resorptions	No. Litters with Resorption	No. Litters	No. Fetal Sites	No. Resorptions	No. Litters with Resorption
Exposed series 1 . . .	15	94	5	4	11	68	9	4	12	93	25	8	19	150	61	16
Exposed series 2 . . .	6	38	9	4	8	50	15	5	10	59	10	3	5	36	10	3
Control	23	221	6	5	15	133	8	4	18	168	7	4	17	160	7	5

300 days of age showed fetal resorption, and these older rats almost always showed multiple resorption (Figs. 2 and 3). Such an increase in resorption incidence correlated with age was not found in the controls examined. In the rats of series 2 there was evidence of increased resorption before age 200 days, which may have been due to incomplete acclimatization at time of gestation. Since exposures did not begin until 125 days of age, it is possible that mating was carried out successfully, as indicated by the 96.2 per cent fertility, but that the deleterious effects of the exposures soon became manifest.

Repeated laparotomies did not reduce the number of fetuses found *in utero*, nor did they show any correlation with the occurrence of abnormal resorption (Table 5). A high resorption ratio was found in exposed females above 250 days of age which had had only 1 operation. Such results indicated that resorption occurred

TABLE 5

FETUSES AND RESORPTIONS OF RATS SUBJECTED TO REPEATED LAPAROTOMY

	Laparotomy No.														
	1			2			3			4			5		
	Age (Days)	No. Alive	No. Resorptions	Age (Days)	No. Alive	No. Resorptions	Age (Days)	No. Alive	No. Resorptions	Age (Days)	No. Alive	No. Resorptions	Age (Days)	No. Alive	No. Resorptions
Control:															
1.....	175	7	1	214	9	0	251	9	0	288	9	0	332	8	1
2.....	180	9	0	215	7	0	258	8	0	293	5	0	342	7	1
3.....	180	9	0	215	9	0	257	9	0	281	10	0	350	9	0
4.....	177	8	0	235	4	3	270	9	0	310	9	0	362	8	0
5.....	136	6	0	161	7	0	191	8	0	250	9	0	.....	.....	.....
Exposed series 1:*															
1.....	150	8	1	195	8	0	252	8	2	286	6	2	321	7	1
2.....	133	3	0	219	3	0	270	9	0	304	5	3	335	9	0
3.....	146	6	0	176	8	0	206	8	0	231	4	0	276	8	0
4.....	192	3	0	308	7	3	364	4	6	.....	.....	.....	.....	.....	.....
5.....	274	6	0	307	3	4	370	2	6	.....	.....	.....	.....	.....	.....
6.....	192	3	0	308	7	3	364	4	6	.....	.....	.....	.....	.....	.....
7.....	216	8	0	307	4	3	335	4	7	.....	.....	.....	.....	.....	.....
8.....	219	2	2	283	1	6	.....	.....	.....	.....	.....	.....	.....	.....	.....
9.....	181	6	1	224	9	0	.....	.....	.....	.....	.....	.....	.....	.....	.....
10.....	225	5	3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Exposed series 2:†															
1.....	177	8	0	214	2	5	249	3	5	285	7	3	.....	.....	.....
2.....	173	3	1	218	8	2	262	10	0	295	7	0	.....	.....	.....
3.....	214	5	0	249	4	0	288	2	5	364	5	2	.....	.....	.....
4.....	170	3	5	204	4	0	300	8	0	.....	.....	.....	.....	.....	.....
5.....	279	10	0	314	7	0	360	10	0	.....	.....	.....	.....	.....	.....
6.....	168	7	1	211	7	2	.....	.....	.....	.....	.....	.....	.....	.....	.....
7.....	175	6	2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

* Exposed to 18,000 feet simulated altitude, 4 hours daily, since 14 days of age. (No operations were performed during first pregnancy.)

† Exposed to 18,000 feet simulated altitude, 4 hours daily, after age 125 days. (No operations were performed during first pregnancy.)

independent of any influence of postoperative complications.

In following the day-to-day bodyweights of the exposed rats in the preliminary experiments, there were eight periods when the typical body-weight changes indicating pregnancy were observed, but none of the animals produced living young. These cases were considered as evidence of loss of a complete litter by fetal resorption. In only one of the 86 laparotomies performed on exposed rats was there a case of all the fetuses being resorbed. The great majority of the resorptions observed were in advanced stages of regression, which points to the early stages of gestation as being very critical. Additional evidence indicating that failure is typically early in gestation is found in the relatively small number of abortions that occurred. There were only three instances of stillbirth of full-term young; they occurred at ages 105, 239, and 345 days. Only 1 litter was aborted at altitude. The only other abortion found was a small necrotic fetus expelled by a rat during handling (Fig. 4). The size of this specimen suggested a case of early fetal death.

The living fetuses lying adjacent to resorption sites were fixed and studied histologically. Such important structures as the liver, the allantois, the yolk sac, and the placenta were examined. No abnormalities were found (Fig. 5). The possibility of the largest of the fetal resorption sites offering some evidence for the failure was also considered. Fifteen of these large resorption sites were studied, and in every case the embryo was histolyzed, making it difficult to discern the various well-known organ systems. It was found that the placenta in most of these fetuses was not severely altered; the principal point of failure was found around the periphery of the placental labyrinth. Marked necrosis of the placenta in this area was found in all the 15 cases examined (Fig. 6), and, as the necrosis was extended in more advanced resorption sites, the entire outer portion of the placenta was involved (Fig. 7). The early stages in the necrosis of this tissue may well have caused the death of the fetus. Specimens were observed in which the entire antimesometrial portion of the gestational sac was filled with the disorganized remains of the fetus (Fig. 8).

An interesting finding was the occurrence of a large amount of hemosiderin in the placental labyrinth of 4 specimens. This material appeared as fine granules extending from the allantois to the edge of the trophoblast in masses running parallel to one another. Other localized groups of larger granules were also scattered about this area. Such material was not found in any other region of the placenta. The pigment was probably of fetal origin, suggesting breakdown of the hemoglobin. It was readily taken up by the syncytial tissues adjacent to the capillaries of the labyrinth.

Considerable quantities of blood accumulated in the uterine lumen at most of the resorption sites, giving them a purplish cast (Fig. 3). Much of this blood apparently originated from the histolyzed vessels of the fetus and in some measure from the hemorrhages in the lateral margins of the placenta.

There was no evidence of infection in the uterine tissues or in the fetal sites. The breakdown of the tissues of the fetus was accompanied by considerable lipoidal accumulation in the uterine lumen. Increased lipoidal accumulation in the tissues of the various areas of the gestational sac was associated with the impending resorption. Abnormal quantities of lipoid were also observed in the tissue of the subplacentalis and particularly in the

giant cells of the trophoblast. In the more advanced stages of resorption (Fig. 9), the remains of the fetus were difficult to find and the placenta appeared as a disorganized mass. The proximity of the blood supply probably enabled the placenta to persist until the fetal resorption was complete. In the resorption of the tissue and lipoid of the decidua in cases of implantation failure and early fetal death (Fig. 10), the impression was gained that the products of disintegration were directed toward the uterine vessels in the region of attachment of the mesometrium to the uterus (Fig. 11).

Survival of the offspring of exposed rats was studied in the 94 litters of the preliminary study and in a large number of controls. The poor survival found among the exposed rats (Table 2) was emphasized when a comparison was made with the observations of survival in 1,529 litters of controls. It was found that 89.7 per cent of the control young born survived until weaning at 21 days, whereas only 67 per cent of the young in series 1 and 60 per cent of the young of series 2 survived through this same period. The high mortality in the exposed rats was related in some measure to faulty lactation. The undernourished young were quite vulnerable to cannibalism and trampling during the first 10 days of life. It was found that the rats that did survive developed in the same manner as those which had been exposed since 14 days of age. Six litters were born at altitude, and five were normal deliveries.

In the analysis of the blood of representative rats throughout the tests, it was found that, after the exposed rats passed 100 days of age, the hematocrit of the males leveled off at approximately 78 per cent and the females at 70 per cent (controls, 50 per cent). The hemoglobin concentration showed corresponding high values of 22.6 gm/100 cc for males and 19.8 gm/100 cc for females (controls, 15.2 gm/100 cc). Such results indicated a considerable acclimatization response on the part of the hemopoietic systems of both sexes, with the males probably being affected by the altitude to a greater degree than the females.

DISCUSSION

It was found that short daily exposure to 18,000 feet simulated altitude adversely affected the reproductive ability of rats. The number of rats that mated was below normal, and there were abnormalities in implantation and gestation. The faulty reproduction was not due to structural changes in the primary sex organs, as was the case at altitudes above 18,000 feet (Gordan *et al.*, 1943; Dalton *et al.*, 1945; Walton and Uruski, 1946; Altland, 1949). The reproductive alterations were due to other factors, probably an endocrine imbalance or a general constitutional debility related to the daily change in oxygen tension and barometric pressure.

Evidence for failure of male sex hormones was observed in the weight reduction of the seminal vesicles and the diminished volume of seminal fluid. Decreased seminal volume was an important factor in reproductive failure in experiments conducted at 19,000 feet simulated altitude (Shettles, 1947) and at 25,000 feet (Altland, 1949). Disturbance in the endocrine system in the male was also reported in another study (Gordan *et al.*, 1943), in which it was shown that the anterior hypophysis of rats had increased gonadotropic potency after discontinuous exposure to 25,000 and 27,000 feet. In the present study interference with the female sex hormones was strongly indicated by the gestational abnormalities found in exposed rats.

The exposure to 18,000 feet did not alter the weight of the rats exposed only

as adults; this finding substantiates similar results reported by Reynolds and Phillips (1947). The slight weight loss in the males exposed at 14 days of age was not found related to any reduction in vitality or with faulty nutrition. All rats were given unrestricted access to water and Purina laboratory chow and to a weekly supply of greens. No difference was noted in the amount of food consumed by exposed and control rats. At autopsy no abnormalities of the digestive tract were found. By way of contrast, in unpublished experiments with rats exposed to 25,000 feet, marked intestinal hemorrhages were rather common.

The impaired reproduction is probably an expression of an insidious constitutional debility that developed during repeated exposures to high altitude. It is well known that pulmonary and circulatory changes, such as increased pulmonary ventilation, respiratory alkalosis, polycythemia, and increased cardiac output occur with the effort to increase the pO_2 (Van Liere, 1942; Houston and Riley, 1947). It is also known that lungs, heart, kidneys, and testes showed pathological changes in rats exposed repeatedly to 25,000 feet simulated altitude (Dalton *et al.*, 1945; Altland, unpublished). In the rats exposed to 18,000 feet no gross pathology of vital organs was found, but the hematocrit and hemoglobin values were only slightly lower than those observed at 25,000 feet. The 18,000-foot level is very near the critical level, since Campbell (1935) reported that mice and rabbits progressively deteriorated and died at 20,000 feet.

This altitude study is the first in which it has been shown that the testis structure was normal but reproductive performance abnormal at a level which induced marked acclimatization changes. It had previously been shown that continuous exposure to 14,260 feet resulted in no change in sex activity in the male (Moore and Price, 1948) and that there were no weight alterations found in the testes of rats exposed 1 hour daily to 18,000 feet simulated altitude (Nelson and Burrill, 1944*a*). It is now possible to state definitely that the changes in the circulatory system due to atmospheric hypoxia occur before any structural alterations in the testis. Such a sequence of events does not necessarily indicate the absence of functional impairment.

A significant finding in this experiment was that the reduced litter size of exposed rats was due to subnormal implantation, increased fetal resorption, and a small degree to cannibalism. The 7.0 implanted ova per litter observed in the exposed rats of series 1 indicated that there was a loss of 2.5 ova per litter when compared with controls. From corpora lutea counts of several exposed rats and from a study of the histology of the ovary, it was indicated that there was no reduction in the number of ovulations. The loss of ova must therefore be attributed to abnormalities between ovulation and implantation, a period which was not considered in this study. Long and Evans (1922) found that a drop from 10.9 corpora lutea in the ovaries of control rats to 9.6 ova in the Fallopian tubes was due mainly to retention in the ruptured follicles. Brambell (1948) reported that in wild rabbits 10.2 per cent of the ova, as determined by corpora lutea counts, were lost before implantation. He pointed out that degenerating ova or degenerating blastocysts were rarely recovered because of their rapid elimination or disintegration.

The occurrence of fetal resorption in 26.9 per cent of the controls and in 48.5–55.1 per cent of the exposed litters leaves no doubt that this high incidence of gestational failure was a very important factor in the reduction of litter size. This finding was further emphasized by the

frequent occurrence of multiple resorption in the exposed rats. The occurrence of faulty gestation in rats exposed to altitudes above 18,000 feet was recently reported by Moore and Price (1948) in pregnant rats exposed 7 hours daily to 21,000 and 22,000 feet.

In the controls of this study there were 9.5 implantation sites per uterus, and, since resorptions accounted for only 0.4 sites per uterus, it must be concluded that abortion and cannibalism accounted for the low figure of 7.6 young per litter at birth. Cannibalism and stillbirths were of secondary importance in the reduction of litter size in exposed rats.

The absence of an abnormal resorption ratio in the rats exposed from 14 to 250 days indicated that they were favorably acclimatized during this period, but this advantage was soon lost after this time. The marked increase in fetal resorption between 300 and 365 days was probably not evidence of premature aging, since almost all the exposed rats lived more than 2 years.

The finding that four or five successive operations upon a single rat had no deleterious effect upon the breeding performance confirmed the findings of Nicholas (1925), who reported that reoperated rats gave rise to normal litters. Long and Evans (1922) also reported that carefully conducted operations seldom interrupted the onset of the next estrous cycle in rats.

Since no histological abnormalities were found in the placenta, yolk sac, allantois, and liver of the fetuses fixed before death, it was necessary to depend entirely upon the histopathological observations of the resorbing fetuses to offer evidence for the cause of the failure. In all the sites studied, the fetus was markedly disintegrated. The only definite cause for fetal death was found in the placenta, which, although reduced only slightly in size, showed enough marginal necrosis to account for the death of the fetus. It is likely that insufficient oxygenation was a cause of the fetal failure. Similar placental necrosis was found to be the cause of death in cases of spontaneous fetal resorption in the Albany strain of rats (Burack *et al.*, 1939). Infection, necrosis, and hemorrhage of the placenta had been associated with fetal resorption in vitamin A deficiency. (Mason, 1935). In these hypoxia experiments no uterine infections were found which could have affected the placental or fetal tissues. Further indication that vitamin deficiencies are not involved in the gestational abnormalities caused by hypoxia was found by making comparison of this work with that of Evans and Burr (1927). In vitamin E deficiency the entire litter was commonly destroyed, and fetal death was due to underdevelopment of the mesoderm and deficiencies of the extra-embryonic membranes. In the altitude tests, loss of a complete litter was rare, and no predisposing fetal abnormalities were found related to fetal death.

The results of this present investigation showed that a large proportion of the cases of gestational failure due to high-altitude exposure was probably due to critical alterations during and immediately following implantation. Many small fetal sites were found in the rats examined during the last week of pregnancy, and the impression was gained that these fetal deaths occurred very early. Such an interpretation was supported by the histological examination of many of these fetal resorption sites and decidual regression sites associated with implantation failure. The observation of only four cases of abortion and the complete absence of maternal death during pregnancy and parturition were unexpected, since other investigators have reported such occurrences to be common in

cases of reproductive failure at high altitude (Loewy and Heller, 1933; Sundstroem and Michaels, 1942; Monge, 1943).

Increased cannibalism among exposed rats undoubtedly resulted in the destruction of some newborn young, as indicated by the low ratio of young per litter in the preliminary experiments, when contrasted to the much higher ratio of living fetuses per uterus obtained by laparotomy. Cannibalism was of secondary importance in the reduction of the litter size.

SUMMARY

Breeding performance was studied in 131 rats exposed to 18,000 feet simulated altitude for 4 hours daily. There was a significant reduction in rate of reproduction in both sexes. The cause of subnormal reproduction was not due to any structural alteration in the primary sex organs. An observed reduction in the seminal vesicle/body-weight ratio of exposed males and abnormal gestation in exposed females indicated endocrinal imbalance. Constitutional debility, associated with the sustained influence of circulatory and respiratory adjustments induced by repeated exposure to high altitude, was considered a possible cause of altered reproduction.

Observations of the uterine contents of 218 rats in late pregnancy by laparotomy or autopsy revealed that reduced size of litters of exposed rats was due to a decreased number of successful embryonic implantations and to an increase in occurrence of fetal resorptions. In the rats exposed only as adults, faulty gestation was observed throughout the experiment; in those exposed since 14 days of age, fetal resorption was not found to be of any significance until after 250 days. Multiple resorptions were common, and the presence of many small fetal sites in late stages of gestation pointed to the prevalence of implantation failure and early fetal death. Resorption of complete litters was observed in only six cases. There were four fetal abortions and no maternal fatalities during parturition. Cannibalism of newborn young was found, but it was of secondary importance in the reduction of litter size.

Histological examination of growing fetuses lying adjacent to fetal resorption sites failed to show any structural deficiencies. Gestational sacs which gave evidence of resorption invariably showed necrotic fetuses in which the well-known organ systems were difficult to identify. The placenta was usually not altered except for marginal necrosis and hemorrhage. Such placental alteration was considered to be a probable cause of fetal death through the consequent hypoxia.

This study showed that in exposed rats there was a marked increase in the hematocrit (females, 40.0 per cent; males, 56.0 per cent) and hemoglobin (females, 31.5 per cent; males, 48.6 per cent) values and that these increased values persisted as long as the exposures were continued.

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PLATE I

FIG. 1.—Uterus of exposed rat (age 345 days), showing two full-term fetuses in each horn.

FIG. 2.—Uterus of exposed rat (age 235 days), showing multiple resorption.

FIG. 3.—Uterus of exposed rat (age 283 days), showing one living full-term fetus and multiple resorption.

FIG. 4.—Abortion. This dead fetus (mother age, 302 days) was expelled while mother was being weighed. × 2.

FIG. 5.—Gestational sac of exposed rat (near term). This fetus was alive at time of fixation and was lying next to fetal resorption sites. Note normal structures. Eosin, methylene blue stain. × 7.

FIG. 6.—Section through gestational sac of dead fetus. Tissue is necrotic at lateral margins of placenta and normal in the central and mesometrial areas. Eosin, methylene blue stain. × 7.

FIG. 7.—Advanced stage of fetal resorption. Lateral margin of placenta completely degenerate. Eosin, methylene blue stain. × 7.

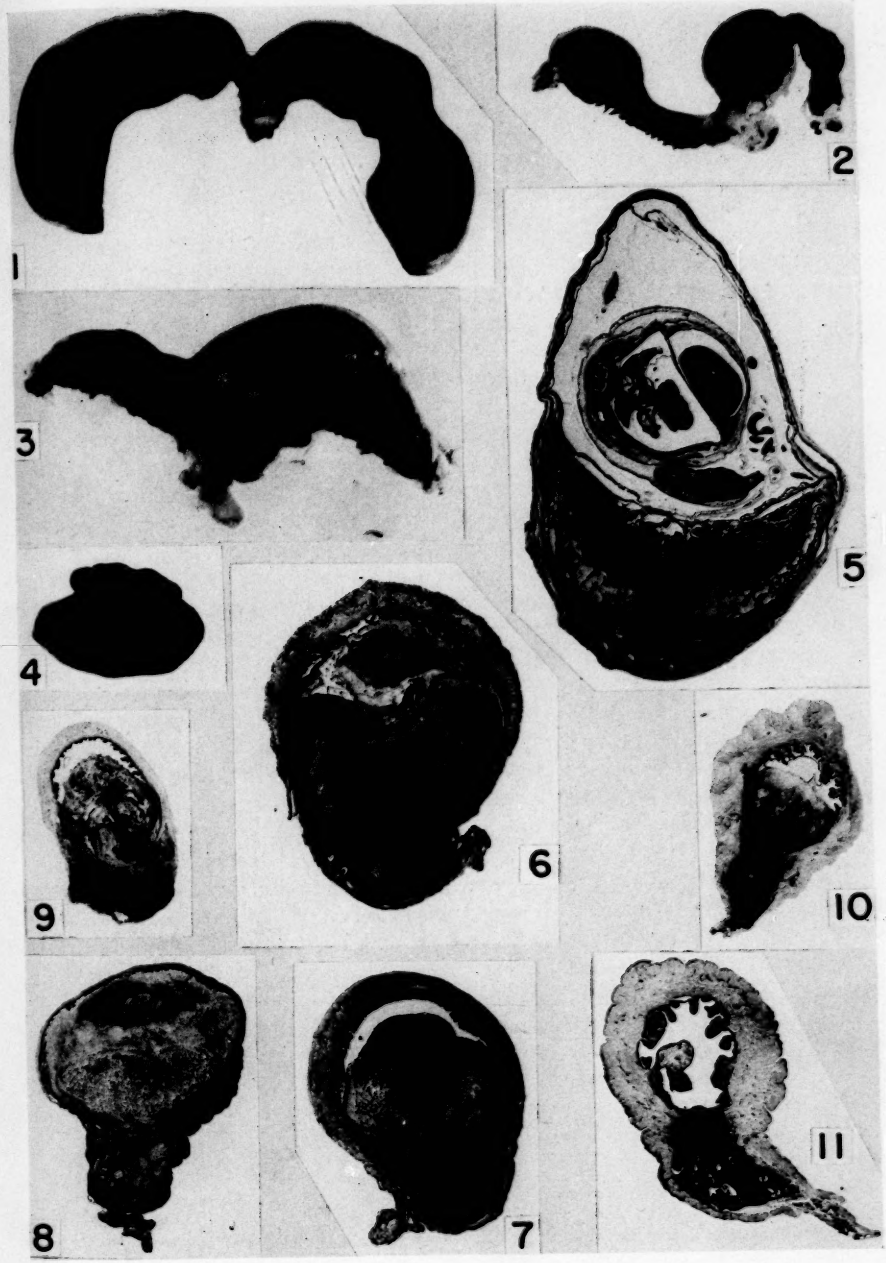
FIG. 8.—Accumulation of degenerative products of fetus in antimesometrial area. No details of embryonic structure distinguishable. Eosin, methylene blue stain. × 7.

FIG. 9.—Degeneration and resorption of fetus and placenta nearing terminal point. Structure of placenta no longer discernible. Oil red O stain (frozen section). × 7.

FIG. 10.—Section through uterus, showing decidual regression following implantation failure. Dark streaks are lipoid masses. Oil red O stain (frozen section). × 7.

FIG. 11.—Uterus of control rat following parturition, showing accumulation of lipoid, including lipoid of metrial gland, in vicinity of uterine vessels in mesometrium oil red O stain (frozen section). × 7.

PLATE I



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THE MODIFICATION OF DEVELOPMENTAL PATTERNS IN THE SAND DOLLAR WITH MALEIC ACID[1]

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FOR the last ten years, whenever conditions permitted, the author has spent the summers either at Pacific Grove or at Woods Hole, working on the problem of control of echinoderm development through the use of external agents. Many chemicals have been used; and, commonly, entirely different ones, within certain effective concentrations, produced similar effects (inhibition of cleavage, excess mesenchyme, exogastrulation, etc.). But this was not always so. LiCl and NaCNS (in Ca-free sea water) before fertilization have exactly opposite effects on the sand-dollar egg, in that lithium produces entodermization while thiocyanate causes ectodermization. The author (Rulon, 1941*a*, 1946) has suggested that this difference in action of the two agents may be due largely to a more rapid recovery, with an increased scale of organization, of the eggs from NaCNS than from LiCl on return to sea water and fertilization. Since data on the actual effects of these different agents on prefertilization metabolism, etc., were lacking, this suggestion has never been entirely satisfactory, although it may well be the correct one.

The author is in complete agreement with the theory that over-all quantitative activity gradients in the developing egg and embryo are very important factors in determining form and proportion during the earlier developmental stages (Child, 1941, p. 172) and that it is through these activity gradients (by differential inhibition, recovery, conditioning, etc.) that external agents act in the modification of developmental patterns. It has seemed for some time, however, that much could be added to the general problem of control of developmental processes if one should deal with agents which have specific effects on metabolic processes rather than those in which the exact action is not known other than that they inhibit or stimulate physiological activity.

In the uncleaved echinoderm egg a gradient of activity (shown by the oxidation and reduction of certain dyes and other methods) is found to extend from the animal to the vegetal pole. This is followed in the late blastula by a second gradient with a high point of activity at the extreme vegetal region.[2] Appearing at the same time, or slightly later, a ventrodorsal gradient can be detected (Child, 1936*a*; 1941, p. 134). That these gradients are important as developmental patterns appears obvious in the light of later development. Although these three gradients are all activity gradients, the morphogenesis under the influence of each finds different expres-

[1] The writer wishes to express his gratitude to Dr. L. R. Blinks, director of the Hopkins Marine Station, Pacific Grove, California, for providing the necessary facilities for this work. To the Graduate School of Northwestern University the writer also wishes to express his thanks for material support given this work.

[2] In a personal communication Dr. Child points out that, while this second gradient appears in reduction of indicators in the sand dollar, he could not observe it with primary oxidation in normal development until invagination occurs.

sion. Could this be because of the activity of different enzyme or enzyme systems in the different gradient fields?

It seems not unreasonable to assume that many enzymes and enzyme systems are involved in the polar activity gradient and that the differential activity of the gradient may be tied in with the activity of such factors. Also, may not other gradients, such as the ventrodorsal, involve, quantitatively or qualitatively, enzyme systems different from those of the polar gradient?

During the past few years the biochemists have developed tools which may be of much use in the solution of some of the problems which confront the developmental physiologist. These tools are in the nature of specific enzyme inhibitors (see Singer, 1945, for review). Do substances which inhibit the iron-porphyrin enzymes have the same effects on developmental patterns and produce modifications similar to those that inhibit other enzymes or enzyme systems?

This paper deals with the first of a series of investigations by the author on the effects of enzyme inhibition on the developing sand-dollar egg. It is an attempt to probe deeper into the underlying factors of physiological gradients and thereby add to the existing knowledge of developmental dynamics.

MATERIAL AND METHODS

This series of investigations was confined to the eggs of the sand dollar, *Dendraster excentricus*. These large, clear eggs could be handled with a medicine dropper with a minimum of injury. The adults were dredged from Monterey Bay during the summer of 1948 in considerable numbers and maintained in the laboratory in aquaria with running sea water. After being washed in fresh water, the animals were opened with scissors, and only the females with ripe, red ovaries, copiously exuding eggs, were used. To prevent contamination by body fluids, the eggs were taken into a medicine dropper in thick masses and transferred to sea water. After having been washed several times with sea water, the eggs were fertilized by adding a few drops of sperm suspension. Since most experiments were performed near the middle of the breeding season, fertilization was usually 95–100 per cent.

The test agent was maleic acid, a substance which is known to inhibit the activity of succinic dehydrogenase, possibly by combination with free sulphhydryl groups, and glyoxalase (by combination with the coenzyme glutathione).[3] It was prepared in isotonic strength and diluted with sea water to the concentrations used in the experiments. The developing eggs were kept in covered finger bowls out of direct sunlight on a bench in the laboratory. All the experiments were conducted at one end of the aquarium room at the Hopkins Marine Station, and numerous records showed that the temperature varied throughout the course of this work by no more than 1° from 18° C.

NORMAL DEVELOPMENT

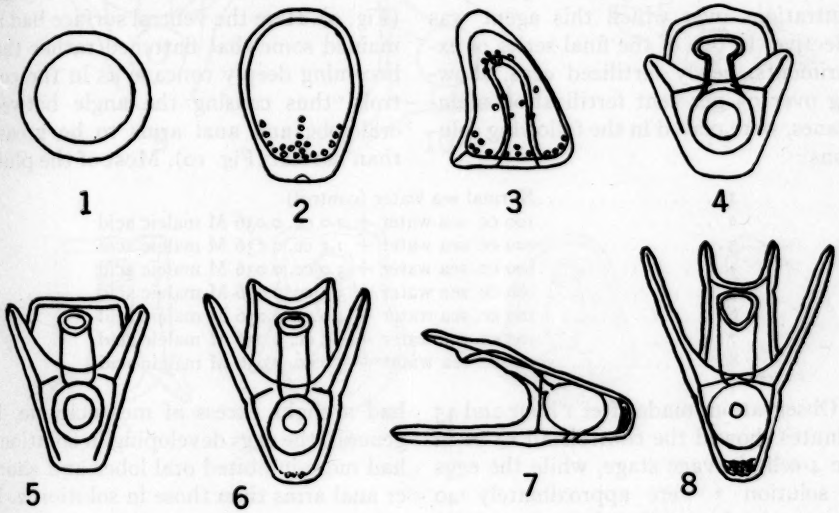
The normal development of the egg of *Dendraster* has been described elsewhere (Child, 1940; Rulon, 1941*a*), but for comparative purposes it is desirable that brief attention be given it here. Also the experiments here described were conducted at a slightly higher temperature (18° C.) than those previously mentioned.

Cleavage is total, and by 6 hours after fertilization the middle blastula stage is

[3] Since the presence of succinic dehydrogenase is not known for these eggs, any effects of maleic acid may be due to its reaction with thiol groups.

reached (Fig. 1). At this time the walls of the blastula are smooth, although still rather thick, and movement through ciliary action has not yet begun. The fertilization membrane is still intact. (Many experiments have been performed by the author and others with embryos at this stage.) By 12 hours after fertilization the larvae have gained cilia and have broken

the anal arms are beginning to make their appearance in basal-lateral positions of the ventral side. By 30 hours (Fig. 4) the ventral surface has become more concave, the anal arms are longer, with fairly well-developed skeletal spicules, the oral lobe has begun to take shape, and coelomic pouches are quite evident.



FIGS. 1–8.—Normal development of the sand-dollar larva from 6 to 60 hours after fertilization at a temperature of approximately 18° C.

from within the encapsulating fertilization membrane. They are now said to be "free-swimming" and are for the most part aggregated near the surface of the culture. They have become elongated in the polar axis, and the walls have become much thinner except for the vegetal region. Primary mesenchyme has appeared, and there may be a slight trace of invagination (Fig. 2). Twenty-four-hour larvae are bilateral, with the ventral surface flattened and the archenteron of considerable length and directed toward the future stomodeal region of the ventral surface (Fig. 3). At this stage

Thirty-six hours after fertilization finds a young pluteus with the stomodeum opening into the gut, ventral surface more deeply concave, anal arms at approximately half their final length and with oral lobe beginning to narrow but as yet with no oral arms (Fig. 5). Six hours later (42-hour pluteus, Fig. 6) the anal arms are longer, there is a definite narrowing of the oral lobe, and the oral arms are appearing. The 60-hour pluteus is well developed, with long anal and oral arms (Figs. 7 and 8). In general, this state was the oldest and most advanced studied in this work. At about this time

the larvae commonly begin to die, since no food is given and the cultures begin to grow stale at this temperature.

THE ACTION OF MALEIC ACID ON DEVELOPMENTAL PATTERNS

1. CONTINUOUS EXPOSURE TO MALEIC ACID FOLLOWING FERTILIZATION

Numerous experiments were performed in determining the range of concentrations over which this agent was effective. In one of the final series of experiments, newly fertilized eggs, showing over 95 per cent fertilization membranes, were placed in the following solutions:

1 . . . . . Normal sea water (control)
2 . . . . . 100 cc. sea water + 2.0 cc. 0.036 M maleic acid
3 . . . . . 100 cc. sea water + 2.5 cc. 0.036 M maleic acid
4 . . . . . 100 cc. sea water + 3.0 cc. 0.036 M maleic acid
5 . . . . . 100 cc. sea water + 3.5 cc. 0.036 M maleic acid
6 . . . . . 100 cc. sea water + 4.0 cc. 0.036 M maleic acid
7 . . . . . 100 cc. sea water + 4.5 cc. 0.036 M maleic acid
8 . . . . . 100 cc. sea water + 5.0 cc. 0.036 M maleic acid

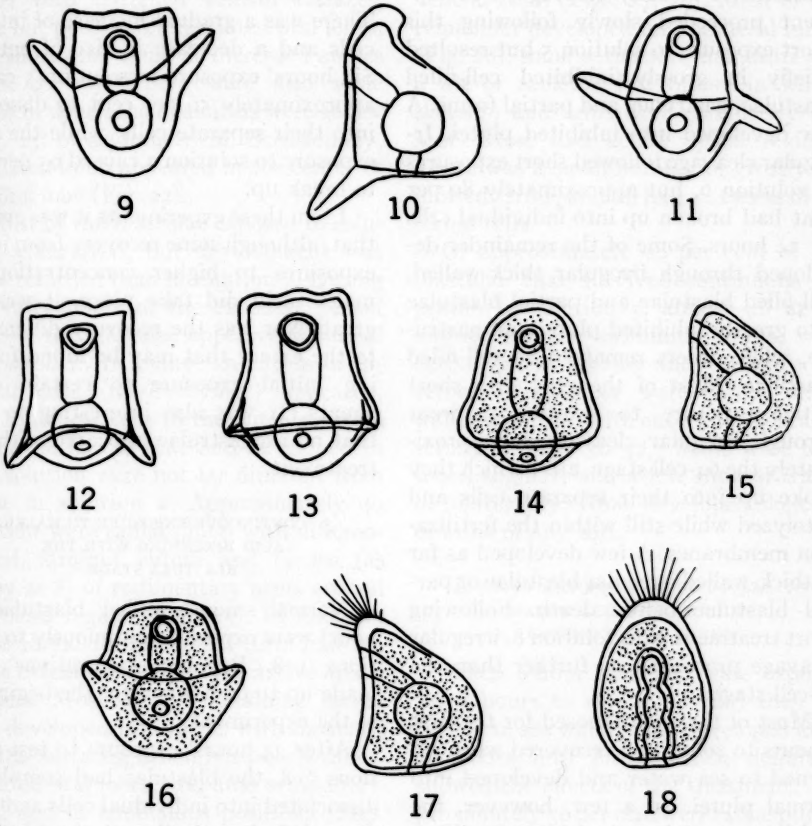
Observations made after 1 hour and 45 minutes showed the controls all to be in the 4-cell cleavage stage, while the eggs in solution 2 were approximately 40 per cent in the 4-cell and 60 per cent in the 2-cell stages. In solution 3 approximately 10 per cent were in the 4-cell and 90 per cent in the 2-cell stages. In solution 4 approximately 10 per cent were in the 4-cell stage, 85 per cent in the 2-cell stage, and 5 per cent still uncleaved. Most of the eggs in solution 5 were in the 2-cell stage, but cleavage was commonly irregular and abortive. In solution 6 only 5 per cent of the eggs had cleaved at all, and these had cleaved abortively, while in solution 7 only 1 per cent showed evidence of cleavage. In the uncleaved eggs it was usually possible to see two nuclei. There was no cleavage of eggs in solution 8, and nuclei were indistinct.

Observations were made of control and test eggs at 6, 12, 24, 48, and 60 hours. Development proceeded normally in the control (Figs. 1–8) but ranged from only slight inhibition in test solution 2 to complete suppression in solution 8. By 60 hours a few of the larvae developing in solution 2 were almost normal, but most were plutei with poorly developed oral lobes and anal arms shortened and at increased angles (Fig. 9). Here the ventral surface had remained somewhat flattened rather than becoming deeply concave as in the controls, thus causing the angle between oral lobe and anal arms to be greater than normal (Fig. 10). Most of the plutei had a slight excess of mesenchyme. In general, the eggs developing in solution 3 had more-inhibited oral lobes and shorter anal arms than those in solution 2. In many, no anal arms at all appeared, and when they did appear they were often asymmetrical and askew (Fig. 11). The ventral surfaces were much flattened (Figs. 12–15), and there were commonly excesses of internal cells (Figs. 14–15). A number of grossly inhibited cell-filled forms that had not progressed past the gastrula stage were present. Most of the eggs in solution 4 developed into little more than cell-filled gastrulae with flattened ventral surfaces. Rudimentary anal arms appeared in some (Fig. 16), but most were completely devoid of skeleton (Figs. 17–18). A few radial cell-filled gastrulae with active apical tufts and well-developed entera (Fig. 18) appeared with this treatment.

The eggs in solution 5 cleaved slowly and irregularly but did not go beyond mid-cleavage stages. By 24-hours, approximately 50 per cent had broken down into separate cells or cell fragments. Cleavage occurred eventually in

2. SHORT EXPOSURE TO MALEIC ACID IMMEDIATELY FOLLOWING FERTILIZATION

Developing eggs were removed from the various test solutions after certain intervals of time and were returned to normal sea water. Following the initial



FIGS. 9–18.—Larvae that have been exposed continuously to graded concentrations of maleic acid, beginning shortly after fertilization.

about 80 per cent of the eggs in solution 6 but was highly irregular and did not go beyond the 8-cell stage. The eggs in solution 7 never gave more than a few beginning cleavages, while there were none in solution 8.

treatment with maleic acid, they were usually observed at 6- or 12-hour intervals up until 60 hours.

Eggs exposed for 1 hour and 45 minutes to solutions 2 and 3 developed into normal plutei on return to sea water.

With such short exposures to solution 4, most of the eggs developed into normal plutei, but there were a few with inhibited oral lobes and anal arms and some grossly inhibited cell-filled forms. There were also a few partial forms which had resulted from separation of the individual blastomeres in early cleavage. Development proceeded slowly following this short exposure to solution 5 but resulted chiefly in grossly inhibited cell-filled blastulae, gastrulae, and partial forms. A few developed into inhibited plutei. Irregular cleavage followed short exposures to solution 6, but approximately 80 per cent had broken up into individual cells by 24 hours. Some of the remainder developed through irregular thick-walled, cell-filled blastulae and partial blastulae into grossly inhibited plutei and gastrulae, while others remained as cell-filled blastulae. Most of the eggs with short initial exposure to solution 7 went through irregular cleavage to approximately the 64-cell stage, after which they broke up into their separate cells and cytolyzed while still within the fertilization membranes. A few developed as far as thick-walled irregular blastulae or partial blastulae before death. Following short treatment with solution 8, irregular cleavage proceeded no further than the 32-cell stage.

Most of the eggs exposed for the first 6 hours to solution 2 recovered when returned to sea water and developed into normal plutei. In a few, however, the oral lobes were broad and poorly differentiated. With 12 hours' initial exposure to this solution, a few were almost normal, but most developed into plutei with broad oral lobes (undifferentiated or partly differentiated), flattened ventral regions, and shortened anal arms, which were sometimes asymmetrical. A number of grossly inhibited cell-filled forms also appeared. With initial exposures of 6 and 12 hours to increasing concentrations of maleic acid, the degree of inhibition increased. Oral lobes failed to differentiate. Ventral regions remained flattened, became convex, or finally failed completely to appear. Anal arms became progressively shorter and at increased angles. There was a gradual increase of internal cells and a decrease in size of enteron. Six hours' exposure to solution 5 caused approximately 50 per cent to dissociate into their separate cells, while the same exposure to solution 7 caused 95 per cent to break up.

From these experiments it was evident that, although some recovery from initial exposures to higher concentrations of maleic acid did take place, it was not great. Nor was the recovery differential to the extent that may be found following initial exposure to certain other agents. It was also interesting to note that no exogastrulae appeared with this treatment.

3. CONTINUOUS EXPOSURE TO MALEIC ACID BEGINNING WITH THE BLASTULA STAGE

Normal smooth-walled blastulae (6-hour) were exposed continuously to solutions 1–8. Regular observations were made up to 60 hours after the beginning of the experiment.

After 24 hours' exposure to test solutions 6–8, the blastulae had completely dissociated into individual cells and were discarded. Practically all the blastulae exposed to solution 2 survived the experiment and, although developing somewhat more slowly than the controls (solution 1), showed at 60 hours a number of interesting modifications. Between 30 and 40 per cent were completely radial (Figs. 19–20) with differentiated entera extending almost to the apical ends. Cir-

cles of arms with short skeletal spicules were around the bases. These forms were usually near the bottom of the culture dish and were characterized by extremely rapid whirling movements. Most of the remaining larvae developing under the conditions of this experiment were plutei with flattened ventral surfaces, wide but poorly differentiated oral lobes, and short anal arms at increased angles (Figs. 21–22). There were also some forms in which the anal arms were askew (Fig. 23) or asymmetrical. Occasionally, a ciliated knob appeared in the center of the oral lobe (Fig. 22).

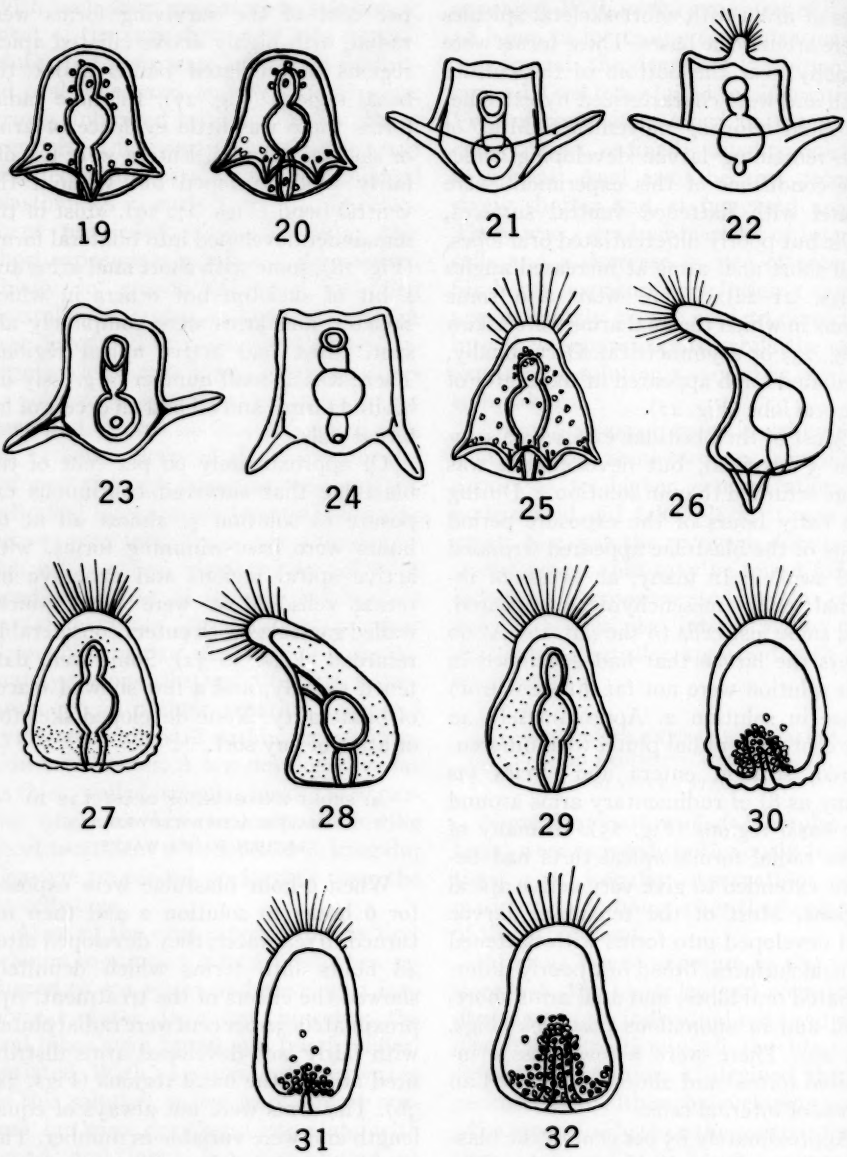
Most of the blastulae exposed to solution 3 survived, but development was more retarded than in solution 2. During the early hours of the exposure period some of the blastulae appeared wrinkled and swollen. In many, an excess of internal cells (mesenchyme?) appeared, and some lost cells to the outside. At 60 hours the larvae that had developed in this solution were not far different from those in solution 2. Approximately 40 per cent were radial plutei with differentiated, straight entera and circles (as many as 8) of rudimentary arms around the basal regions (Fig. 25). In many of these radial forms, apical tufts had become extended to give very active apical regions. Most of the remaining larvae had developed into forms with flattened ventral surfaces, broad but poorly differentiated oral lobes, and anal arms shortened and in anomalous positions (Figs. 24, 26). There were a few grossly inhibited forms, and almost all showed an excess of internal cells.

Approximately 85 per cent of the blastulae exposed to solution 4 survived the experiment, although the later blastula stages commonly became irregular and lost cells both to the outside and into the blastocoel. At 60 hours, approximately 50 per cent of the surviving forms were radial, with highly active ciliated apical regions and ciliated bands about the basal regions (Fig. 27). In these radial forms there was little evidence of arms or skeletal spicules. Entera were usually fairly well developed but without the ventral bend (Figs. 27, 29). Most of the remainder developed into bilateral forms (Fig. 28), some with short anal arms and a bit of skeleton but others in which skeleton and arms were completely absent. Most had active apical regions. There was a small number of grossly inhibited forms, and all had an excess of internal cells.

Of approximately 60 per cent of the blastulae that survived continuous exposure to solution 5, almost all at 60 hours were free-swimming forms, with active apical regions and excessive internal cells. Most were fairly thick-walled gastrulae with entera considerably retarded (Figs. 30–32). Some were flattened slightly, and a few showed traces of bilaterality. None developed skeleton or arms of any sort.

4. SHORT EXPOSURE OF BLASTULAE TO MALEIC ACID FOLLOWED BY RETURN TO SEA WATER

When 6-hour blastulae were exposed for 6 hours to solution 2 and then returned to sea water, they developed after 48 hours into forms which definitely showed the effects of the treatment. Approximately 50 per cent were radial plutei, with fairly well-developed arms distributed around the basal regions (Figs. 34, 36). The arms were not always of equal length and were variable in number. The remainder graded from forms that were almost radial (Fig. 35) up to almost normal bilateral plutei, with many having increased angles to the anal arms and broad oral lobes (Fig. 33). Almost all

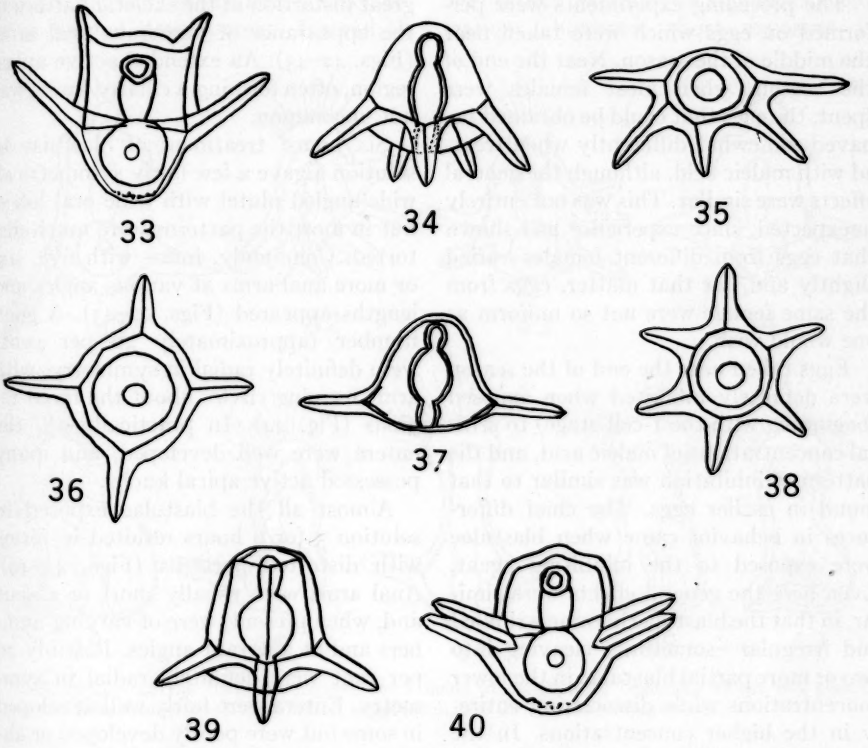


FIGS. 19–32.—Larvae that have been exposed continuously to graded concentrations of maleic acid, beginning at the mid-blastula stage.

had well-differentiated entera. The extended highly ciliated apical region, commonly found with continuous exposure beginning at the 1-cell stage, was usually absent. Only a few grossly inhibited forms resulted from this treatment.

curred, but most had widened oral lobes and anal arms at increased angles. A few grossly inhibited forms were present.

Blastulae exposed for 6 hours to solutions 4 and 5 showed much recovery when returned to sea water. Between 50 and 60 per cent developed into radial



FIGS. 33–40.—Larvae that were exposed for 6 hours to maleic acid, beginning at the mid-blastula stage, and were then returned to sea water.

Six hours' exposure of blastulae to solution 3 gave approximately 60 per cent radial forms spinning rapidly about in the culture. In general, these forms were similar to those resulting from treatment with solution 2, but a few had active apical regions, and often more internal cells were present. Some apparently quite normal bilateral plutei occurred, but the surrounding arms were often unequal in size. A considerable number of bilateral plutei appeared. Some were almost normal, but most had broad oral lobes and anal arms at increased angles. Often the anal arms were duplicated (Fig. 40). There was a small number of grossly inhibited forms, and many, including the radials, had an

excess of internal cells. A few small forms (resulting from fission or the partial breaking-up of blastulae during the period of exposure) were found with this treatment.

5. OTHER EXPERIMENTS WITH MALEIC ACID

The preceding experiments were performed on eggs which were taken near the middle of the season. Near the end of the season, when most females were spent, the eggs that could be obtained behaved somewhat differently when treated with maleic acid, although the general effects were similar. This was not entirely unexpected, since experience had shown that eggs from different females varied slightly and, for that matter, eggs from the same female were not so uniform as one would wish.

Eggs taken near the end of the season were definitely inhibited when exposed (beginning with the 1-cell stage) to critical concentrations of maleic acid, and the pattern of inhibition was similar to that found in earlier eggs. The chief differences in behavior came when blastulae were exposed to the inhibiting agent. Even here the general effects were similar, in that the blastulae became wrinkled and irregular—sometimes cleaving into two or more partial blastulae in the lower concentrations while dissociating entirely in the higher concentrations. In the lower concentrations plutei developed with widened oral lobes and with anal arms at increased angles, duplicated and often askew. The chief differences were that fewer (sometimes as low as 5 per cent) radial plutei developed with these later eggs and the developmental patterns were often much more distorted.

In one of the final series of experiments, 6-hour blastulae were exposed for 6 hours to solutions 1 (control), 3, 4, and 5, after which they were returned to sea water. After 48 hours the blastulae exposed to solution 3 had developed chiefly into plutei with widened, but fairly well-developed, oral lobes and anal arms at increased angles (Fig. 33). In many of the remaining forms the anal arms were askew (Fig. 41), while a number showed great distortion of the skeletal pattern in the appearance of numerous anal arms (Figs. 42–43). An extended active apical region, often forming a ciliated knob, was not uncommon.

Six hours' treatment of blastulae to solution 4 gave a few fairly symmetrical, wide-angled plutei with wide oral lobes, but in most the patterns were much distorted. Commonly, forms with five, six, or more anal arms at various angles and lengths appeared (Figs. 42–43). A good number (approximately 20 per cent) were definitely radial in symmetry, with arms forming circles about the basal regions (Fig. 44). In practically all, the entera were well developed, and many possessed active apical knobs.

Almost all the blastulae exposed to solution 5 for 6 hours resulted in forms with distorted patterns (Figs. 45–49). Anal arms were usually short or absent and, when present, were of varying numbers and at aberrant angles. Possibly 20 per cent were definitely radial in symmetry. Entera were fairly well developed in some but were poorly developed or absent in others. Almost all had extremely active and extended ciliated apical regions, which often appeared to be developing almost independently.

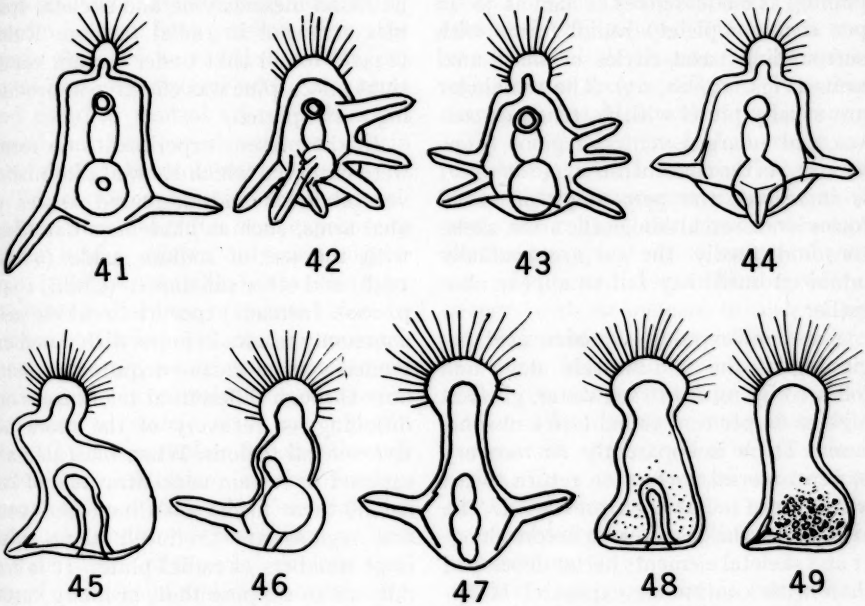
DISCUSSION

On continuous exposure to critical concentrations of maleic acid the eggs of the sand dollar develop in a manner similar to that found by exposure to a number of different agents. Cleavage and the developing embryo show more or less in-

hibition, depending on the concentration of the agent. With low concentrations the oral lobe is prevented from differentiating, the anal arms are shortened, and, although there is less development of skeleton, an excess of internal cells appears. The ventral side of the animal does not usually become deeply concave, as in normal development. Instead, it

centrations, the development of arms and skeleton may be entirely prevented, and in extreme cases a ventrodorsal differential may not appear at all.

Exposure for short periods immediately after fertilization and followed by return to sea water has little effect in the low concentrations. In higher concentrations, however, short exposure is



FIGS. 41–49.—Larvae that were exposed for 6 hours to maleic acid, beginning at the mid-blastula stage, and were then returned to sea water. (Eggs taken near the end of breeding season.)

not only remains flattened but commonly increases in size, a condition which may lead to an increase in the angle between the two anal arms and between the anal arms and oral lobe. This increase in size of ventral surface appears to be the result of differential tolerance (or conditioning) in which the more active ventral region is able to tolerate (or become conditioned to) the agent sooner and to a greater degree than does the less active dorsal region. With slightly higher con-

quite effective, and the changes produced are in most ways similar to those with continuous exposure to lower concentrations. Differential recovery from the effects of maleic acid takes place to a limited extent, as is shown by an increase in size of ventral surface and an increase in the angle of the anal arms.

Continuous and short exposures to the proper concentrations of maleic acid, beginning at the mid-blastula stage, give interesting results. From these ex-

periments it seems clear that mid-blastula stages are less susceptible to lethal concentrations of the agent than are early cleavage stages. A degree of tolerance, apparently, may be acquired (if not already present) by the blastula to concentrations that inhibit and eventually stop cleavage. Continuous exposure to low concentrations of maleic acid beginning at 6 hours gives as high as 30–40 per cent completely radial forms with surrounding basal circles of short anal arms (Figs. 19, 20, 25). The remainder are usually plutei with flattened or convex and enlarged ventral regions (Figs. 21–24). As the concentration of the agent is increased, the percentage of radial forms increases, although the arms, skeleton, and, finally, the gut are gradually inhibited until they fail to appear altogether.

Six hours' exposure to maleic acid, beginning at the mid-blastula stage and followed by return to sea water, gives as high as 60 per cent radial forms in some cases. There is apparently no recovery of ventrodorsal pattern on return to sea water, once it has been prevented. The arms about the basal region become longer and skeletal elements better developed than with continuous exposure. It appears that a short interval following the mid-blastula stage is most critical in the development of bilaterality and that strong inhibition by maleic acid at that moment (before acclimation to the agent takes place) is important in preventing bilaterality.

The production of radial forms in echinoderm embryos is not new. Many different agents applied externally have prevented the development of ventrodorsality (Child, 1936*b;* 1941, p. 201). In most cases, however, it has been necessary to use such high concentrations and long exposure periods that the radial forms were little more than cell-filled blastulae, gastrulae, or exogastrulae, with little evidence of arms, skeleton, or ectodermal differentiation. There have been some exceptions. In work with sodium azide, Child (1948) produced a number of radial forms in *Strongylocentrotus*. Many of these had ciliated bands encircling the basal regions, and in some he found mesenchyme and skeletal spicules arranged in radial fashion. Rulon (1941*b*) found that under certain conditions pilocarpine was effective in producing radial plutei.

In the present experiments no forms were observed which showed diminished ventral areas and decreased angles to anal arms, such as have been described with the use of sodium azide (Child, 1948) and other substances (Child, 1941, p. 200). Instead, exposure to maleic acid commonly results in forms with much extended ventral areas—acquired presumably through differential tolerance, conditioning, or recovery of the more active ventral regions. When blastulae are exposed to certain concentrations of the agent, these forms with increased ventral regions are produced along with large numbers of radial plutei. It is not difficult to suppose that, in many cases, the scale of organization of the ventral region may be so increased that it (the ventral region) extends completely around the larva. Since the anal arms arise at the anal border of the ventral region in normal development, their appearance all the way around suggests that conditions approaching that of the anal border of the ventral region may have been determined all the way around. It may be appropriate to call this phenomenon a "ventralization," since it is comparable in many respects to the "animalization" found to take place through the use of NaCNS in Ca-

free sea water before fertilization (Lindahl, 1933, 1936; Rulon, 1941*a*, 1946) but involves the ventrodorsal rather than the polar gradient. If this is actually a process of "ventralization," intermediates between strictly bilateral and radial plutei should be present in the cultures. Unfortunately, the data are incomplete on this point. A few definitely intermediate forms were noted (Fig. 35), and many more may have been present among the forms classified as radial but with unequal arms or bilateral with extra arms and enlarged ventral areas. It is to be hoped that future work with maleic acid may clarify the problem.

The above explanation for the development of radial plutei from exposure to maleic acid is offered only as a suggestion. It seems not unlikely that the more inhibited radial forms (such as those described by Child [1948; 1941, p. 200] and, for that matter, resulting from treatment with high concentrations or continuous exposure to maleic acid) are brought about by the complete failure (through differential inhibition) in the development of a ventrodorsal differential. Under such conditions there still may be a certain degree of recovery or conditioning of ectodermal and mesenchymal elements and their subsequent orientation to a radial rather than a bilateral pattern.

A condition of "balance" seems to be present in most of the radial plutei produced with maleic acid. The differentiated gut extends straight from the blastopore (anus) without the ventral bend that is found in bilateral forms. A stomodeum was absent in all the strictly radial forms studied. It was also interesting to note that in all the work with maleic acid no exogastrulae were observed. This, of course, does not mean that exogastrulae do not occur with this agent, but it does seem to indicate that the factors concerned with ventrodorsal pattern are more susceptible to this agent than are those concerned with invagination and differentiation of archenteron.

In the radial and certain other forms resulting from exposure to low concentrations of maleic acid, the apical regions develop very little but, with higher concentrations and longer exposure periods, become highly ciliated, thickened, and extended. This development of the apical region is not greatly different from that described by Hörstadius (1935, 1937, 1939) when the animal blastomeres are completely isolated from basal or vegetal regions. In the present experiments it seems not unlikely that the overdevelopment of the apical region may be the result of differential conditioning or recovery (with an increase in scale of organization) of this region while the vegetal region (because of an initial lower activity) remains inhibited. In this connection it is interesting to note that, with the increase and extension of the animal region, there is a decrease in development of the vegetal region, often to the extent of a decrease or obliteration of the archenteron.

Child (1941, pp. 201–2) has pointed out that the polar gradient is much more difficult to obliterate than is the ventrodorsal gradient; yet modifications through differential inhibition, recovery, etc., in the polar gradient are not difficult to obtain with many different agents. Various agents likewise affect the ventrodorsal gradient, and most of the modifications grade from a flattening in low concentrations, to a decrease in size and even obliteration of the ventral surface in high concentrations. All agents, however, do not act in the same way on the ventrodorsal pattern. Pease (1941, 1942*a*, *b*) placed eggs of the sand dollar in steep

concentration gradients of various enzyme inhibitors and found that, while certain ones readily shifted the ventrodorsal axis, others did not. He states that the most inhibited side of an egg in concentration gradients of certain substances tends to become the dorsal side. By this he refers to inhibition relative to the ventrodorsal axis rather than to cleavage, since he found that certain substances inhibited cleavage on the side of greatest exposure without having any effect on ventrodorsality. Pease's experiments seem to be especially important because they indicate a close relationship between ventrodorsality and certain enzymes or enzyme systems. They also show that cleavage phenemona may involve enzyme action that is quite different from that concerned with ventrodorsality.

In discussing axiate pattern, Child (1948) states:

> Since the enzyme inhibitors, KCN and NaN_3, as well as other agents which depress metabolism differentially in one way or another, can progressively decrease and obliterate axiate pattern, it appears more probable that the physiological basis of such pattern is primarily a quantitative, metabolic gradient pattern with a gradient pattern of enzymes as protoplasmic correlate.

This statement would appear to hold equally true for the ventrodorsal pattern. The ventrodorsal gradient is a gradient of activity, and it is in this activity, which is primarily quantitative in nature, that the pattern for future differentiation lies. It also seems likely that this ventrodorsal activity gradient may be modified through inhibition of enzyme correlates which may be different (quantitatively or qualitatively) from those of other gradient systems in the echinoderm egg.

The ventrodorsal gradient cannot be separated entirely from the polar gradient. The same protoplasm is certainly found in both. Yet the graded correlates of each are at approximately right angles to one another. It is to be expected—and, indeed, the work with maleic acid and other agents shows—that agents which affect ventrodorsal pattern also affect polar pattern. But these two patterns are not affected to a comparable degree by all agents. Some affect ventrodorsality more, while others affect the polar pattern more—a condition which may well be related to different correlative factors in the two gradient systems.

SUMMARY

1. Continuous and short exposures of newly fertilized eggs of *D. excentricus* to graded concentrations of maleic acid cause inhibition of cleavage followed by inhibition of oral lobe, anal arms, and ventral surface. Occasionally, the ventrodorsal differential is completely obliterated. Differential conditioning to and recovery from the agent may result in the ventral area's becoming increased and convex with anal arms at increased angles.

2. Continuous and short exposures to the proper concentration of maleic acid, beginning at the mid-blastula stage, often result in a high percentage of radial plutei, as well as bilateral forms with flattened or convex ventral surfaces. Under the proper conditions a circle of arms, varying in number, develops around the base of the radial form, and the enteron, while differentiating into three segments, does not develop the ventral bend.

3. No exogastrulae appeared in these experiments following treatment with maleic acid. With high concentrations

and in certain lots of eggs, the apical regions became thickened, extended, and highly ciliated.

4. Ventrodorsal and polar activity gradients are discussed, and the possibility of differences between these two fundamental patterns being related to enzyme correlates is mentioned.

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DOMINANCE RELATIONS BETWEEN DIFFERENT BREEDS OF DOMESTIC HENS[1]

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SOCIAL hierarchies based on the dominant or subordinate behavior of every individual toward all others of the group have been described for many vertebrates. Status in the hierarchy appears to be decided by the outcome of an active fight or by passive submission of one individual to the threats of another. Psychological and physiological factors, especially endocrine products, which influence this type of behavior have received particular attention. Allee (1942) and Collias (1944) have recently reviewed most of the pertinent findings. Although Howard (1920) and Schjelderup-Ebbe (1935) were both impressed by differences in fighting ability and pugnaciousness among different spe-

[1] I am indebted to Professor W. C. Allee for suggesting the study and for his advice and encouragement which were given throughout the course of the work. I am also indebted to Professor Sewall Wright for his considerable aid with the statistics.

cies of birds and Schjelderup-Ebbe (1922, 1935) by similar differences between individuals of the same species, little work designed to investigate the genetic aspects of social dominance has been reported.

Scott (1942) found consistent differences in the unconditioned, preliminary fighting behavior of three highly inbred strains of mice. Ginsburg and Allee (1942) used these same strains in a study of the social effects of conditioning. They, too, found consistent, unconditioned differences in both the preliminary fighting patterns and the ability of these mice to win interstrain combats. Billingslea (1941) and Hall and Klein (1942) found that rats selectively bred for emotionality under certain experimental conditions were markedly less aggressive toward other rats than were those selectively bred for nonemotionality in the same situations. Other studies, particularly of rats, have linked "aggressiveness" with both physical characteristics (coat color) and other behavior differences, such as wildness. For a review of such studies see Hall (1942). These latter experiments gauged "aggressiveness" on reactions to the observer or inanimate objects rather than on reactions to other animals. Aggressive behavior toward other animals may or may not be linked with "aggressiveness" toward the keeper. Ginsburg and Allee state that their C-57 black mice, which were most aggressive toward other mice, were most pacific toward the keeper; the albinos, the most submissive strain toward other mice, were, on the criterion of biting the experimenter, the most aggressive toward man. Hall and Klein found that rats of their nonemotional strain were more aggressive both toward other rats and toward the keeper than were rats of the emotional strain. The value of genetic investigation of "aggressiveness" directed toward objects other than those which might naturally be attacked appears limited in interpreting natural differences in dominance at the present state of knowledge.

The present study was undertaken in an effort to determine whether differences in social dominance between birds of the same species but of somewhat different genetic backgrounds could be demonstrated. In view of the recognized knowledge of many factors, other than genetic, which make for greater social dominance in chickens, the strong tendency toward a straight-line social hierarchy of the flocks, the absence of definite territoriality, and the availability of distinct breeds, common domestic hens were chosen as the experimental animals.

ANIMALS AND THEIR TREATMENT

For the purposes of this study, sexually mature hens bred to conform to recognized breed standards were obtained from commercial sources or from university flocks.[2] Breeds were chosen to give as wide a range in weights and temperaments as were readily available. Table 1 shows the number of birds and the breeds that were used. It will be noted that the average weights for most breeds are somewhat below standard. In order to insure maximum social interaction among the hens, they were not fed up to their full weights.

Except for three White Wyandottes and three Brown Leghorns, all birds of a given breed were obtained from the same source. For this reason it is necessary to consider the behavior of any breed, as determined by these experiments, to be

[2] I am indebted to Dr. L. E. Card, of the University of Illinois, for supplying me with Rhode Island Red hens and to Dr. L. V. Domm, of the University of Chicago, for providing most of the Brown Leghorns.

the behavior of a given strain of that breed and not necessarily to be typical of the breed as a whole. To simplify the discussion of behavior differences, birds of one breed will be referred to by the breed name throughout the paper. Such treatment is not intended to imply that general breed differences were determined.

Animals received at the laboratory were banded with colored rings, weighed, and penned together as single-breed flocks for a period of at least 3 weeks to allow them to become accustomed to laboratory conditions, the physical aspects of which were described by Allee, Collias, and Lutherman (1939). All birds except the Game and Brown Leghorn hens appeared to become adjusted to frequent observations both from outside and from within their pens and to necessary handling. Even after months at the laboratory, the above-mentioned breeds typically ran to the far end of the pen when it was entered and flew to the tops of the nests which were placed there. When approached, these birds would squawk and run or fly from the observer. In contrast, the other hens learned to approach the observer when he entered with food and to ignore the observer at other times. All hens became adjusted to observations made while the observer remained outside the pen. All birds usually resumed laying within 3 weeks of receipt at the laboratory.

The birds were fed scratch feed at the morning observation period. During the first year they were given a second ration of grain in the evening, but this was discontinued when it was found difficult to control the weights of some of the dominant birds. Mash, enriched with cod-liver oil, was always present. Cabbage was given twice a week. Hoppers containing grit, charcoal, and oyster shell, as well as waterers, were kept filled. During the first year the hens were fed exclusively from hoppers; later, scratch feed was scattered evenly over the floor. Experience showed that this method of feeding reduced the number of pecks and threats by approximately one-fourth, brought about earlier organization of new flocks, and permitted persecuted individuals to maintain their weights better.

The experimental, multiple-breed flocks were all composed of two White Leghorns and one or two of most of the other breeds used. Table 2 gives the identity and breed of every individual in each of these twelve flocks. The flocks were assembled by first securing each member-elect in a small box on the roosts or in a trap nest. Then all birds were placed on the floor as rapidly as possible. In most cases no fights occurred until all birds were on the floor and the observer outside the pen. The peck-order of each flock was established from observations of initial pair-contacts at the time of flock formation and from subsequent daily recordings of pecks and threats delivered and received.

Following the method elaborated by Collias (1943), ranks of the birds based on the number of individuals pecked

TABLE 1

THE BREEDS OF HENS USED IN THE EXPERIMENTS

Breed	Average Weight (In Ounces)	Individuals
Light Brahma	100.0±4.0	5
Rhode Island Red	98.0±3.5	5
White Wyandotte	75.5±2.0	5
White Leghorn	65.0±1.0	15
Brown Red Game*	61.0±2.5	5
Brown Leghorn	44.5±2.5	10
White Cochin Bantam	23.0±0.5	4

* One Game hen was a cross between a Brown Red Game and a Yankee Clipper. She did not differ significantly in weight or behavior from the full-blooded Brown Red Games.

were adjusted to a common scale, which took account of the unequal size of the flocks. For purposes of analysis, reductions of flock size by deaths were ignored in computing adjusted ranks, even when members died before the flock was well organized. This procedure was considered justified, since dominance relationships, established in the first day of flock formation, were maintained until the flock was broken up, except for one case.

Once the multiple-breed flocks became organized as established by reduced pecking pressures and increased tolerances (see Guhl and Allee, 1944), birds from different flocks were tested in staged pair-contacts with hens of different breeds. The technique used was similar to that described by Allee, Collias, and Lutherman (1939), with the exception that no estimate of outcome was recorded. When it became apparent that certain breeds tended to win over others in these staged contacts, the contests were so planned that, as far as possible, no bird was permitted to win or lose more than three times in succession. (Collias reported in 1943 that he was able, in the few cases tested, to reverse the outcome of staged contests between two hens by arranging for the initial winner to lose and the initial loser to win

TABLE 2

INDIVIDUAL HENS USED IN TWELVE FLOCKS*

Flocks	Breeds						
	White Leghorn	Rhode Island Red	Game	White Wyandotte	Brown Leghorn	Light Brahma	White Cochin Bantam
1C	WBW RW	RWB	Y	RY	BW DR	BG	.....
2C	G R_{w1}	PY	B	GW	RB R_b	Y	.....
3C	BW YB	PW	W	PR	RY	.....	.....
1D	B YG	PW	W	RY†	RY	BG	.....
2D	WRW RG	PW	W	PR	RY	O	.....
3D	YB_2 RB	RWB	Y	PR	DR	O	.....
1G	RB YO	DR	Y	PY	BL	BG	B
2G	YB_2 BL	LR	W	RY†	P	.....	Y
3G	B YG	RWB	G	.....	G	BB	R
2H	RB YG	PY	P	R	O	Y	YO
3H	YB_2 YO	DR	W	.....	RB	P	Y
4H	BL O	RWB	G	PY	Y	BB	R

* Identical symbols in the same breed column indicate the same individual.

† The White Wyandotte, RY, became masculinized during the course of the observations. It is omitted from consideration in flocks 1D and 2G throughout the paper and is here included only for the sake of completeness.

several contests with strange birds staged between the first and second encounters of the original opponents.)

During the second year of work the birds were segregated into single-breed flocks for at least 10 weeks and again tested in staged pair-contacts with birds of different breeds. As members of these single-breed flocks, hens met other breeds only at the time of pair-contacts. These staged contests were so arranged that 3 weeks elapsed between any two meetings with the same heterotypic breed.

TABLE 3

THE SOCIAL POSITIONS OF BREEDS IN TWELVE FLOCKS

Breed	Social Positions				
	Alpha	Beta	Gamma	Delta to Psi	Omega
White Leghorn	9	8	4	3	0
Game	2	3	1	6	0
Rhode Island Red	1	1	5	5	0
Brown Leghorn	0	0	1	12	1
White Wyandotte	0	0	0	7	1
Light Brahma	0	0	1	5	4
White Cochin Bantam	0	0	0	0	6

THE SOCIAL ORDERS OF THE FLOCKS

Table 3 summarizes the social positions of the birds grouped according to breed in the twelve experimental flocks. The assigning of these positions is in some cases somewhat arbitrary because of the large number of triangles which existed in these flocks. Where two birds in the same flock each pecked the same number of subordinates, the higher social position was accorded to the dominant bird of the pair. In straight-line hierarchies, which are usual for small single-breed flocks, no two birds peck the same number of subordinates, and the rank of any bird can be indicated by the number of its subordinates.

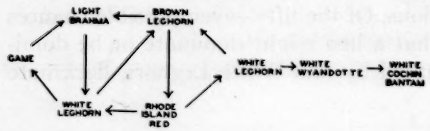
Despite the complications introduced by triangles, certain breeds tended to achieve either very high or very low positions in the social order of their flocks. The two White Leghorns present in each flock tended to hold adjacent social positions. Of the fifty-seven possible chances that a hen might dominate or be dominated by one White Leghorn flockmate and not the other, only seven instances occurred. The correlation coefficient between the ranks of the two White Leghorns in twelve flocks was .912.

The social orders in eight of the twelve experimental flocks were complicated by triangles: two flocks each had one, four flocks had two, one flock three, and the eighth flock, four. Diagram 1 illustrates the most complicated peck-order observed, that of flock 1G. Internal partial reversals of the direction of pecks similar to the relationship existing between the Brown Leghorn, Light Brahma, the more dominant of the White Leghorns, and the Game also occurred in other flocks exhibiting more than one triangle.

These social orders were perfectly stable over periods up to 6 months. Flocks were not kept intact for longer

times. Hens of all breeds except the Bantam, which always held the omega position, were, in some flocks, members of one or more triangles. Workers using single-breed flocks have noted that triangles were relatively rare and that they

DIAGRAM I



THE PECK-RIGHTS IN FLOCK 1G

some birds were moved together into new flocks as they were assembled. In only one case was the dominance relation established between two hens reversed when they were transferred as a pair directly into a new flock. There was no evidence that these remembered relationships played any important role in the occurrence of the numerous triangles.

Table 4 gives the dominance relations of all breeds to all other breeds, omitting those cases in which birds entered new flocks with such relations already established.

TABLE 4

DOMINANCE RELATIONS BETWEEN BREEDS IN TWELVE FLOCKS*

SUBORDINATE BREEDS	DOMINANT BREEDS							
	White Leghorn	Game	Rhode Island Red	White Wyandotte	Brown Leghorn	Light Brahma	White Cochin Bantam	Totals
White Leghorn	.....	7	4	0	1	4	0	16
Game	14	.....	3	3	4	1	0	25
Rhode Island Red	20	6	.....	0	3	3	0	32
White Wyandotte	16	4	7	.....	3	3	0	33
Brown Leghorn	27	6	8	7	.....	2	0	50
Light Brahma	16	8	7	3	11	.....	0	45
White Cochin Bantam	12	6	6	5	6	6	.....	41
Totals	105	37	35	18	28	19	0	.....

* To read the table, read the number directly below the dominant breed and opposite the subordinate breed. For example, read: White Leghorn dominates Rhode Island Red twenty times; Rhode Island Red dominates White Leghorn four times.

tended to straighten out in time (Collias, 1944). Masure and Allee (1934) and Guhl (1945) report that flocks of cockerels show many triangles but that their hierarchies are unstable and many reversals occur. The complicated, but stable, dominance relationships which existed in the present small multiple-breed flocks do not appear to be entirely comparable to the types of dominance relations existing in single-breed flocks.

An examination of Table 2 shows that

Chi squares were calculated, using Yates's correction for each pair of breeds in the table. The theoretical frequencies were taken as half the observed number of dominance relations for each pair of breeds. The calculations shown in Table 5 for White Leghorn–Game dominance relations illustrate the method. Note that Yates's correction results in reducing the observed frequency of dominance for White Leghorns by 0.5 and increasing that of Game hens by the same quantity.

The sum of the chi squares for all pairs of breeds in Table 4 is 101.49; the probability is less than 0.001.[3]

The over-all percentages of dominance calculated from the totals in the margins of Table 4 show certain relationships. The standard errors of the proportions were calculated, and the probabilities of obtaining differences as great as, or greater than, those observed were computed. The White Leghorn percentage of total dominance differed significantly at the 0.01 level from those of all other breeds. The Game hens were more dominant than any of the breeds listed to the right of them, except the Rhode Island Red. Rhode Island Reds, White Wyandottes, and Brown Leghorns did not differ significantly from one another; the deviation of the Rhode Island Red breed from the Brown Leghorn had a *P*-value of 0.054. Rhode Island Reds were more dominant than either Light Brahmas or Bantams. The total dominance percentages of neither White Wyandottes nor Brown Leghorns were significantly larger than that of Light Brahmas; all three were more dominant than the Bantam.

An examination of Table 2 shows that some birds appeared in more than one flock. In order to test whether the later performances of individuals in achieving social rank varied sufficiently from preceding performances for them to be considered as breed representatives in each flock, an analysis of variance was carried out.[4] An adjusted rank was calculated for each bird in each multiple-breed flock of which it was a member, following the method elaborated by Collias (1943).[5] The mean square of ranks of individuals was compared to the mean square of ranks achieved in first flocks by hens of the same breed. Table 6 presents the data used in the calculations. The ratio of the mean square of ranks of all individuals of the same breed to the mean square of the first ranks achieved by hens of the same breed has a probability of more than 0.05 and hence is not considered significant. The individuals varied as much in successive performances as hens of the same breed did from one another.

TABLE 5

AN ILLUSTRATION OF THE METHOD OF COMPUTING CHI SQUARES FOR TESTING THE SIGNIFICANCE OF DATA PRESENTED IN TABLE 4

Breed	Observed Frequency	Calculated Frequency	Difference	Chi Square
White Leghorn.....	13.5	10.5	3	.857
Game.....	7.5	10.5	3	.857
	.....	.....	Chi square=	1.714

Since individuals could be treated as breed representatives in each flock of which they were members, an analysis of variance of the ranks achieved by all hens in all flocks was made in an effort to

[3] The probability value gives the odds of obtaining results as bad or worse through the operation of chance alone. Customarily *P*-values of 0.05 or less are considered to indicate statistically significant differences.

[4] I am indebted to Professor Sewall Wright for supplying me with the appropriate formulas and for suggesting the tabular setup of the data, which differs somewhat from the standard type of table ordinarily used for an analysis of variance.

[5] On the assumption that factors affecting rank are normally distributed, Collias used the following formula to compute adjusted ranks:

Adjusted rank = Inverse probability function of

$$\left(\frac{\text{Number of hens pecked} + \frac{1}{2}}{\text{Number of hens in the flock}} - \frac{1}{2}\right).$$

Since the mean of the distribution is zero, half the hens will hold positive ranks, the other half negative ones.

TABLE 6

A COMPARISON OF THE VARIATION OF THE RANKS OF INDIVIDUALS IN LATER FLOCKS WITH THAT OF THE INDIVIDUALS OF THE SAME BREED IN THE FIRST FLOCKS OF WHICH THEY WERE MEMBERS

Breed Mean of Ranks in First Flocks	$\Sigma(X-\bar{X})^2$ for First Ranks of Hens of the Same Breed	Hen	Rank in First Flock	Rank in Second Flock	Rank in Third Flock	Rank in Fourth Flock	Rank in Fifth Flock	Mean of Ranks of Individual	$\Sigma\Sigma(X-\bar{X})^2$ for Ranks of Individual
			White Leghorn						
0.462	2.0690	B	0.67	0.79	.....	.....	.....	0.73	1.8548
		YG	−0.21	0.37	0.88	.....	.....	0.347	
		YB_2	1.47	1.39	1.47	.....	.....	1.443	
		RB	0.79	0.49	1.53	.....	.....	0.937	
		YO	−0.16	0.79	.....	.....	.....	0.315	
		BL	0.21	0.88	.....	.....	.....	0.545	
			Rhode Island Red						
0.452	0.1356	RWB	0.49	0.37	0.37	0.16	.....	0.350	0.7861
		PY	0.49	−0.49	.....	.....	.....	0.000	
		PW	0.67	0.37	0.21	.....	.....	0.417	
		DR	0.16	−0.37	.....	.....	.....	−0.105	
			Brown Red Game						
−0.193	1.2481	Y	−0.88	−0.37	0.88	.....	.....	−0.123	3.8538
		W	0.67	0.00	0.67	0.37	0.79	0.50	
		G	−0.37	1.53	.....	.....	.....	0.58	
			Brown Leghorn						
−0.787	0.9588	DR	−0.16	−0.37	.....	.....	.....	−0.265	1.4900
		RY	−0.67	−0.79	−1.39	.....	.....	−0.950	
		RB	−1.53	0.00	.....	.....	.....	−0.765	
			White Wyandotte						
−1.135	0.1300	PR	−1.39	−0.37	−0.37	.....	.....	−0.710	0.7696
		PY	−0.88	−0.49	.....	.....	.....	−0.685	
			Light Brahma						
−1.225	0.9770	BG	−1.53	0.21	0.16	.....	.....	−0.387	3.3900
		Y	−1.53	0.16	.....	.....	.....	−0.685	
		O	−1.47	−1.47	.....	.....	.....	−1.47	
		BB	−0.37	−0.37	.....	.....	.....	−0.37	
			White Cochin Bantam						
−1.430	0.0032	Y	−1.39	−1.47	.....	.....	.....	−1.43	0.0050
		R	−1.47	−1.53	.....	.....	.....	−1.50	
Totals	5.5217	.....	.....	.....	.....	.....	.....	.....	12.1493
Degrees freedom	17	.....	.....	.....	.....	.....	.....	.....	37
Mean squares	0.3248	.....	.....	.....	.....	.....	.....	.....	0.3284

determine whether the differences in ranks held by the different breeds were significant. Table 7 gives the adjusted ranks of every hen according to breed; Figure 1 presents the same data in graphic form. (Since some individuals were members of more than one flock, the same individual at different times are in accord with the findings of Douglis (1948). In the present experiment the breed of a hen appeared to set limits on its variability, but, within the range held by its breed, a hen could hold any rank, and its past performance was of little

TABLE 7*

ADJUSTED RANKS OF ALL BIRDS IN THE EXPERIMENTAL FLOCKS

White Leghorn	Rhode Island Red	Game Hens	White Wyandotte	Brown Leghorn	Light Brahma	White Cochin Bantam
1.53	0.67	1.53	0.16	0.16	0.21	−1.39
1.53	0.49	0.88	0.16	0.16	0.16	−1.47
1.53	0.49	0.79	−0.37	0.00	0.16	−1.47
1.47	0.37	0.67	−0.37	−0.16	−0.37	−1.53
1.47	0.37	0.67	−0.49	−0.21	−0.37	−1.53
1.47	0.37	0.37	−0.49	−0.37	−0.37	−1.53
1.39	0.21	0.16	−0.88	−0.49	−1.47	
0.88	0.16	0.00	−1.39	−0.49	−1.47	
0.88	0.16	−0.16		−0.67	−1.53	
0.88	−0.21	−0.37		−0.67	−1.53	
0.88	−0.37	−0.37		−0.79		
0.79	−0.49	−0.88		−1.39		
0.79				−1.53		
0.79				−1.53		
0.79						
0.67						
0.49						
0.37						
0.21						
0.21						
0.21						
0.16						
−0.16						
−0.21						

* Mean square of ranks within breeds is 0.3136; degrees of freedom are 79; mean square of ranks between breeds is 6.6881; degrees of freedom are 6.

number of ranks for a given breed in Table 7 exceeds the number of individuals of that breed listed in Table 1.) The ratio of the mean square of ranks between breeds to the mean square of ranks within breeds has a probability of less than 0.01, indicating that real differences exist between the breeds.

Tables 6 and 7 are of value in demonstrating the great variability in rank achieved both by hens of the same breed and by the same individual in different flocks. The differences in ranks held by

value in predicting the rank that it would hold in a new flock.

The variability within breeds makes it impossible to assign to them averages of rank that are meaningful. In all cases except that of the Bantams the standard deviations are equal to considerably more than half the breed mean computed from adjusted ranks. The relative homogeneity of the Bantams is a result of their always holding the omega position in the flocks. If other breeds whose dominance capacities were more nearly like those of

the Bantams had been used, the Bantams would probably have shown considerable variation in the measurement of this character.

Individuals rarely achieved the same or nearly the same ranks in different flocks. Only four of the twenty-two hens listed in Table 6 held ranks in later flocks which were consistent with those held in the first flock of which they were members. The White Leghorn, YB_2, held the alpha position three times; no familiar flockmates ever moved with this hen into new flocks. (The Game hen, W, was a member of both flocks 2G and 2H, but a period of several months elapsed between the breaking-up of the G flocks and the assembling of the H flocks.) The Light Brahma, O, was the omega bird in two flocks. Only one established dominance relationship was carried over into her second flock. The Light Brahma, BB, twice held the position third from the bottom, and, since, like YB_2, her flocks were of the G and H series, it is improbable that remembered relationships played any role in her second performance. (It might be noted that BB held two dominance relations in her H flock which were the reverse of the relations held with the same birds in her G flock.) The White Leghorn, B, was dominated by one bird in each of her flocks and was each time a member of a triangle. The eighteen variable hens held ranks from omega to alpha; the greatest single range for one bird was 1.9 points on the adjusted scale (Game hen, G, flocks 3G and 4H). In this instance the total possible range for G was 3.00 points.[6]

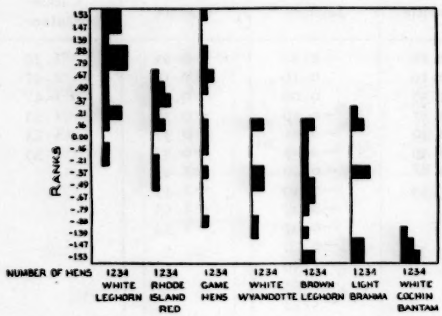


FIG. 1.—Ranks held by representatives of seven breeds in twelve multiple-breed flocks. The widths of the blocks represent the number of hens holding a given rank; the zero line connects blocks where no hens held intervening ranks. An analysis of variance indicates that the difference between breeds is significantly greater than the difference within breeds (see Table 6). For a discussion of the relative dominance relations of one breed to another see page 267.

THE STAGED INITIAL PAIR-CONTACTS

Staged initial pair-contacts between hens are an efficient means of obtaining data relevant to social dominance. In the present study, social rank was always determined by the outcome of the first encounters between every pair of individuals making up the flock. This observation is supported by most workers in the field (see Collias, 1943, 1944). Douglis (1948) reported somewhat different results. She found that, while social orders established at initial contacts did not change, the subordinate hen of a pair sometimes threatened or actually fought with its superior at a subsequent meeting. Since these challenges were not effective in reversing the dominance relations established at the first meeting, the observations of Douglis do not appear to be sufficient to invalidate the initial pair-contact as a technique for measuring the relative social dominance of two strange hens.

A series of 874 contacts were staged be-

[6] The range in adjusted ranks was determined by the number of hens in a flock. Thus in a flock of eight hens the alpha bird would have a rank of +1.53 and the omega bird a rank of −1.53, provided that neither was involved in a triangle.

tween strange individuals of different breeds. Of these, 40 were indecisive, i.e., the observer was unable to distinguish the dominant hen by the behavior of the two birds. The results of the 834 initial contacts which were decisive are presented in Table 8. The table is designed to point up the hierarchy of winning breeds which became evident as the study progressed. Of the twenty-one types of contacts staged, the results of five were inconclusive, since no statistical significance (P was equal to more than 0.05) existed between the difference in the number of wins between the two breeds involved in each of these five types. Probably no importance should be attached to the differences between the White Wyandotte–Bantam and the Game-Bantam successes, as the numbers of contacts are small.

TABLE 8

PAIR-CONTACTS WITH SUBORDINATE BREEDS

Type of Contact	Wins	Losses	P-Values*
White Leghorn:			
vs. Rhode Island Red....	56	18	0.000
vs. Game.....	38	31	0.471
vs. White Wyandotte....	37	7	0.000
vs. Brown Leghorn.....	62	3	0.000
vs. Light Brahma.....	45	5	0.000
vs. White Cochin Bantam.	16	0	0.000
Rhode Island Red:			
vs. Game.....	31	11	0.003
vs. White Wyandotte.....	42	1	0.000
vs. Brown Leghorn.....	42	2	0.000
vs. Light Brahma.....	41	4	0.000
vs. White Cochin Bantam.	17	0	0.000
Game:			
vs. White Wyandotte.....	40	13	0.000
vs. Brown Leghorn.....	51	2	0.000
vs. Light Brahma.....	31	5	0.000
vs. White Cochin Bantam.	12	3	0.039
White Wyandotte:			
vs. Brown Leghorn.....	35	9	0.000
vs. Light Brahma.....	19	16	0.736
vs. White Cochin Bantam.	11	3	0.061
Brown Leghorn:			
vs. Light Brahma.....	26	9	0.007
vs. White Cochin Bantam.	11	7	0.484
Light Brahma:			
vs. White Cochin Bantam.	15	7	0.136

* Using Yates's correction, chi squares were calculated for frequencies of dominance for each pair of breeds with a theoretical frequency of 50 per cent of the number of contests. Probabilities were read for t (= chi) from normal curve tables.

Since the outcome of pair-contacts between strangers determined the social order that existed in the flocks, it was of interest to compare the performance of breeds in the staged pair-contact situation with their performance in achieving dominance in flocks. Fourfold tables giving the frequencies with which a breed dominated or was dominated by another breed in both pair-contacts and flocks were set up. Chi squares, again using Yates's correction, were computed to test the significance of the differences between the two types of performance. Table 9, showing data taken from Tables 4 and 8, illustrates the method.

TABLE 9

AN EXAMPLE OF A FOURFOLD TABLE USING DATA FROM TABLES 4 AND 8 FOR THE CALCULATION OF CHI SQUARES (YATES'S CORRECTION IS OMITTED)

Breeds	Frequency of Dominance in Flocks	Frequency of Dominance in Pair-Contacts
White Leghorn.....	20	56
Rhode Island Red....	4	18
	24	74
	Chi square = 0.38	
	P = 0.538	

The techniques used presented the birds with somewhat different conditions in the pair-contacts as contrasted to the first meetings of new flock members. It will be recalled that in forming a new flock all the strange birds were placed together in the pen at the same time; in staging pair-contacts, two hens met as

strangers in an otherwise empty pen. Furthermore, some hens were always familiar with the pen in which a new flock met, and in nine of the twelve flocks some of the hens of the new flocks were familiar with one another. Despite these differences, the correspondence between breed dominance in flocks and in initial encounters between strangers was good. In only four of the twenty-one comparisons did P-values of less than 0.05 indicate that the performances in the two situations were not comparable. These values are: Rhode Island Red–Game, $P = 0.049$; Rhode Island Red–Brown Leghorn, $P = 0.012$; Rhode Island Red–Light Brahma, $P = 0.031$; and Game–Brown Leghorn, $P = 0.001$.

THE ROLE OF EXPERIENCE

Some of the behavior notes recorded at the time that the pair-contacts were staged indicated that the hens might in some cases behave toward their opponents on the basis of general behavior patterns built up toward the opponent's breed rather than respond to the opponent as an individual. An example of this type of data is the history of the Game hen, W. In the first multiple-breed flock of which she was a member, W dominated both of her White Leghorn flockmates. In five pair-contacts staged with strange White Leghorns, W attacked the strangers and won each contest after an active fight. W was transferred to a new flock in which she was subordinate to both White Leghorns. While a member of this latter flock, W was paired with four more White Leghorns which she had not met previously. She lost each of these contests, three times by default when she ran from her opponent and remained in one corner of the pen, although the Leghorn involved had not obviously threatened her. At the same time, W was winning all her pair-contacts with breeds other than White Leghorn.

That a hen might actually mistake one member of a breed for another under certain conditions is illustrated by the following case, variations of which were observed to occur six times with different animals. The Game hen, B, was paired with a strange Rhode Island Red. A hard fight ensued, which B won. B was returned to her home flock, where a social superior, another Rhode Island Red, approached her. B immediately attacked her superior and won the fight that followed. The Rhode Island Red remained apart from B for many minutes after this fight. Later the Rhode Island Red met B while wandering around the pen. B avoided her; the Rhode Island Red raised her head, and B avoided again. This was followed by a peck delivered to B by the Rhode Island Red. Continued observation showed that the social order of the flock had been in no way disturbed by B's temporary victory over her superior; B continued to take the subordinate role at each subsequent meeting.

Analysis of the results of staged pair-contacts between hens which were members of multiple-breed flocks and those which were members of single-breed flocks indicated that, in general, experience in the home flock did not statistically affect either the results of the pair-contacts or the total number of active fights that ensued. Three exceptions occurred. In the following types of pair-contacts the second-listed breed won significantly more contests when all hens were members of single-breed flocks: White Leghorn–Rhode Island Red; White Wyandotte–Light Brahma; and White Wyandotte–Brown Leghorn.

Experience in the home flock did have

a decided effect on the tendency of a bird to submit passively or to fight if attacked. If threatening action were initiated by a hen of the same breed as a dominant in the home flock, the bird so threatened usually submitted without resistance; if the threatening hen was of the same breed as a known subordinate, the chances that the threatened hen would actively fight with its attacker were much greater. Table 10 gives the total number of active fights and passive submissions which resulted from different types of threats in pair-contacts between birds familiar with the different breeds in the home flocks. Because of the strong tendency of certain breeds to achieve certain positions in the social orders with respect to other breeds and the tendency of the more dominant hen to threaten first, the numbers of examples of each type are disproportionate. Note that 347 of the total 508 contacts were of Type I, while only 89 were of Type III.

In order to test the possibility that experience might play a dominant role in the achievement of high social position in the multiple-breed flocks, these were assembled in series of threes, with the different breed representatives having different backgrounds. In the C series (see Table 2) all hens lacked multiple-breed flock experience, although the White Leghorns were somewhat older and had been at the laboratory for at least a year prior to their use in these flocks. In the D series, the White Leghorns had no prior membership in flocks with other breeds, and the other hens had all been members of C flocks. In the G series new representatives of breeds other than White Leghorn were used where possible; two White Leghorns were unfamiliar with the other breeds. The H flocks were assembled from both experienced and inexperienced birds; however, all had been confined to single-breed flocks for at least 10 weeks prior to the assembling of the H flocks. The behavior of the hens indicated that this was sufficiently long for all G flock dominance relations to be forgotten. Four dominance relations between former G flockmates were reversed.

There appeared to be little difference in the performance of the various breeds with these different experiential backgrounds. Table 11 gives the breakdown by flock series for the social positions of the White Leghorns.

TABLE 10

ACTIVE FIGHTS AND PASSIVE SUBMISSIONS RESULTING FROM DIFFERENT TYPES OF THREATS

Type	Active Fights	Passive Submissions	Per Cent of Active Fights
I*.....	39	308	11.2
II†.....	18	54	25.0
III‡....	42	47	47.2

* Type I: Threatening hen of same breed as familiar dominant.

† Type II: Threatening hen of breed not represented in home flock.

‡ Type III: Threatening hen of same breed as familiar subordinate.

TABLE 11

THE SOCIAL POSITIONS OF THE WHITE LEGHORNS GROUPED BY SERIES

Flock Series	Social Positions of White Leghorns				
	Alpha	Beta	Gamma	Delta to Psi	Omega
C.....	2	2	1	1	0
D.....	3	2	0	1	0
G.....	2	2	1	1	0
H.....	2	2	2	0	0

BREED DIFFERENCES IN BEHAVIOR DURING PAIR-CONTACTS

Certain striking differences in behavior were noted during pair-contacts which appeared to be related to the breeds of the birds. All hens except the Game spent some time in preliminary circling of the opponent before they attacked. The Game hens either attacked immediately or stood rigid for as long as 2 minutes and then attacked in a quick rush. The median latent period for Game hen attacks was 3.8 seconds. Only the relatively less dominant White Wyandottes had a median latent period prior to attack which approached that of the Game—10.4 seconds. All other breeds showed median latent periods of between 20 and 35 seconds.

Both the Game hens and the Rhode Island Reds were remarkable for the persistence which they displayed in active fights with one another. Fights between other breeds seldom lasted for more than 8 seconds. Fights between Game and Rhode Island Red hens usually lasted for several minutes, and both birds appeared definitely tired at the end of such a fight. During a long fight the birds would interrupt the actual combat with short rests. These were usually initiated by the Game hen's displaying a form of behavior never observed in the other breeds. The Game hen would work its head under the wing or among the breast feathers of the Rhode Island Red and apparently lean against the larger bird. This usually inhibited pecking on the part of the Rhode Island Red until the Game bird's head was again visible, when the fight would continue.

Members of all breeds except the Game were observed at one time or another to lower one wing and circle the opponent. This was usually accompanied by frequent halts, when the circling bird would pick up pieces of wood shavings from the floor. This type of behavior strongly resembles courtship "waltzing" and tidbitting, as seen in cocks. However, the movements were much slower than is usual for the cock, and they were not accompanied by any vocalization. Schjelderup-Ebbe (1913) noted that cocks sometimes displayed this type of behavior before attacking another cock. In the present experiments the slower a hen was to attack, the more likely she was to "waltz" or pick up objects from the floor in her beak.

DISCUSSION

Factors which affect social dominance in hens are many, and their interactions are complex. Among the physiological factors known to contribute to the achievement of dominance are (1) greater weight, (2) maturity, (3) slightness of molt, and (4) an increase in the amount of male hormone present. On the psychological side, familiarity with the area in which an encounter takes place, presence of flockmates, and high social rank increase the chances for success in a meeting with a stranger. There is some evidence (Allee and Collias, 1940; Collias, 1943) that a successful series of encounters with strangers favors the winning bird in subsequent encounters and that a series of losses handicaps an animal. Such data for fowl are by no means so extensive or convincing as are similar data for mice (Ginsburg and Allee, 1942; Beeman and Allee, 1945).

Despite the evidence that social dominance is achieved through success in fighting or bluffing and that definite breeds of English fighting cocks were well known by the seventeenth century, there has been very little work designed to investigate genetic differences in natural dominance between various breeds of

chickens. Fennell (1945) reported that game cocks invariably won encounters with cocks of domestic breeds. The present study was made in an effort to determine whether differences in breed could be correlated with differences in dominance which might be genetic in origin. Tables 3, 4, and 8 demonstrate the observed differences in dominance between given strains of different breeds. It remains to examine the possibility that factors other than genetic might have been important in determining these differences.

In general, differences in weight were not important determinants of differences in dominance. The most dominant of the breeds examined, the White Leghorn, is a bird of medium size. The most subordinate breed was the very light, White Cochin Bantam. However, the latter's submissiveness appeared to be a result of temperament rather than of weight. These bantam hens were relatively successful in pair-contacts with both the Brown Leghorns and the Light Brahmas; the latter breed was the heaviest of those used. Since this study was completed, two White Cochin Bantams have been paired with immature New Hampshire pullets and cockerels, against which they have been successful, although the younger birds outweighed them by 32 or more ounces. The most aggressive of these Bantams, R in Table 2, was carefully conditioned upward by wins against the New Hampshires. Then, under conditions arranged to be especially favorable to her, this Bantam was allowed to meet the Game hen, P, which had been conditioned downward by a series of losses. R won the peck-right over P at this meeting and has retained it for 4 months in a flock in which R pecks only P and another Bantam. The fact that the Bantams rarely became involved in active fights, where their small size might have been a handicap, also argues for the importance of factors other than weight differences in determining their lack of social success.

In one type of pair-contact the greater size of the winning bird did appear to be important. Over 43 per cent of the Rhode Island Red–Game contacts resulted in active fights. The Rhode Island Reds won 52 per cent of these; in every case the losing Game hen submitted only when she was no longer able to stand. The greater endurance of the Rhode Island Reds may have been a function of their greater weight. Ordinarily hens did not fight vigorously enough for one of them to be either seriously wounded or exhausted, and to the observer the beaten animal appeared frightened rather than physically injured.

The effect of maturity in these experiments is difficult to evaluate. Except for the White Leghorns, three Game hens, and seven Brown Leghorns, the ages of the birds were unknown. All were in good egg production when received and continued to lay throughout the course of the study except for normal physiological pauses, such as those accompanying periods of molt or broodiness. In the case of the White Leghorns, six one-year-old hens achieved as high social positions in their flocks and were as successful in staged interbreed pair-contacts as were three-year-old birds. Since the other hens of known age were hatched within 3 months of one another, comparisons cannot be made.

Collias (1943) found that slightness of molt was the greatest single factor contributing to success in initial encounters between moderately inbred White Leghorn hens. In the present study the winner of an encounter tended to be a bird of a given breed regardless of the states

of molt of the two contestants. The Rhode Island Reds, for example, had a long molting period and consequently were more often rated as showing greater molt than their opponents in staged pair-contests. Nevertheless, these birds won a higher percentage of their encounters than any other breed except the White Leghorns. However, pair-contacts were not staged with animals which were molting severely or which appeared ill either as a result of molt or for other reasons. New flocks were assembled only when most of the hens were in good plumage; this factor was thus fairly well controlled during the establishment of dominance relationships in the flocks.

Nothing is known about the relative amounts of male hormone secreted by the different hens. Within a breed comb size can be used as a rough indicator of the relative amounts of androgens present in the chickens. There appear to be genetic differences in comb sensitivity between breeds. Dorfman (1948) found the combs of White Leghorn chicks to be more sensitive to testosterone propionate applied locally than were the combs of Rhode Island Reds or Barred Rocks. In regard to the observed differences in dominance between the various breeds, it is possible that certain of them may secrete more male hormone or that their nervous systems may be more sensitive to its effects.

Disease has been reported to have different effects on the achievement and maintenance of dominance (Allee, Collias, and Lutherman, 1939; Douglis, 1948). Twelve birds died during the course of the present study. Only one of these—the Rhode Island Red, PW, of Flock 2D—had lost status and then to only one bird in the flock, the Game, W. Birds were not tested in pair-contacts if they showed any symptoms of disease or general lack of vigor. Positive diagnosis of disease was not possible; post mortem examinations of eight of the carcasses revealed lesions which might have resulted from avian leukosis,[7] and, of the other four, multiple lesions in almost every organ, which might have indicated general tubercular infections.

The psychological effects resulting from familiarity of territory or presence of flockmates were controlled during the staged pair-contacts but not when new flocks were formed. At the time of the first meetings of the hens in all flocks, except those of the C series, some birds were familiar with the area of meeting, some with one or more flockmates, and some with both area and flockmates. Such cases were too diverse to permit an evaluation of the possible effects of these factors. The agreement for most pairs of breeds between dominance in the flock and success in pair-contacts indicates that the two samples were drawn from the same universe. It seems probable that any psychological advantage resulting from familiarity with area of meeting or flockmates tended to be masked by the effects of factors common to both the staged pair-contacts and the initial meetings of flockmates.

Social rank in the home flock was closely associated with success in staged pair-contacts. Over 80 per cent of those contests which took place when the birds were members of multiple-breed flocks were won by the hen holding the higher social position. Collias (1943) found social rank to have only a low positive correlation ($.262 \pm .084$) with success in initial encounters staged between White Leghorn hens. In the present study high social rank and success in initial encoun-

[7] I am indebted to Dr. Saul Narotsky, of Michigan State College, for post mortem examinations of two of these hens.

ters were both associated with certain breeds. When the hens were segregated into flocks of one breed, the association of success with breed was clearly marked. For example, in interbreed pair-contacts staged at this time, the omega hen of an all-White Leghorn or an all-Game flock almost invariably won encounters with the alpha bird of all-Light Brahma or all-Brown Leghorn flocks.

The effects of earlier wins and losses on the outcome of pair-contacts staged between strange hens has not been extensively studied. Collias (1943) observed reversals of success in two cases in which the original winners of the contacts were subjected to a series of losses and the original losers to a series of wins between the first and second meetings of the pairs. On the other hand, Allee, Collias, and Lutherman (1939) describe a hen treated with testosterone propionate which won all encounters with strangers while under the influence of the drug but then began to lose all encounters after treatment was discontinued and the physiological effects dissipated. Among the series of encounters reported in this paper, seven instances occurred in which the winner of a staged contest had lost, and the loser won, three or more encounters immediately preceding the one in question.

Although the evidence for conditioning to success in hens is conflicting and our knowledge not sufficient to resolve these conflicts, it seems worth while to comment on the possibility that such conditioning may have affected the results reported here. In this study the method of conducting the pair-contacts minimized any conditioning effect on birds of intermediate dominance but could not control such effects on very dominant or very subordinate hens. Dominant hens usually had a long series of wins prior to any particular contest, subordinate hens a series of losses, and intermediate birds a series of alternating wins and losses. In contests between two individuals, both of which were members of either relatively dominant or relatively subordinate breeds, the total numbers of previous wins or losses did not influence the outcome of the immediate contest. When dominant breeds met subordinate breeds, the earlier wins of the one and losses of the other may have increased the probability for success of the more dominant breed.

It was mentioned earlier that hens which were threatened by strangers of the same breed as a known subordinate tended to fight the stranger more frequently than if it were of the same breed as a known dominant. In these cases the attacker, if subordinate to the breed attacked in its home flock, tended to lose the fight. Over 98 per cent of all pair-contacts were won by the bird initiating them. When the initiator was subordinate in its home flock to the breed of the bird it threatened, it won only 35 per cent of the encounters. If a threatened hen was subordinate in its home flock to a bird of the same breed as its attacker, the attacker won 90 per cent of the contests, regardless of its own home-flock status relative to the breed attacked. Hens were usually slower to threaten strangers of the breed of a known dominant and did so only in 26.4 per cent of all encounters. Although the differences are not significant statistically, except in three cases, hens of dominant breeds won fewer pair-contacts when they were members of single-breed flocks than when they were members of multiple-breed flocks. These facts may be an indication of greater natural dominance of some breeds or only chance effects. However, they may also indicate that hens

may respond to strange individuals with behavior appropriate to their relations with known birds which resemble the strangers in appearance. Individual histories (see p. 272) give support to the latter theory, which is now under investigation in this laboratory.

In the light of the possibility that conditioning to breed may take place, it becomes important to consider the possible effects of the presence of two White Leghorns in every flock. Although the variability of ranks of birds of this breed was considerable, there was a strong tendency for the White Leghorns in each flock to hold adjacent positions in the social hierarchies. The correlation coefficient of .912 between ranks of the two White Leghorns in the twelve flocks is probably higher than could be accounted for by similarities in dominance resulting from breed alone. Even where triangles complicated the social structures, the White Leghorns tended to peck or be pecked by the same flockmates. Reference to Diagram 1 on page 266 will show that in Flock 1G one member of the pair was involved in three triangles, the other in one, and yet both pecked the Brown Leghorn, White Wyandotte, and Bantam and were pecked by the Rhode Island Red and Light Brahma. Only the Game hen split the social dominance of these two White Leghorns. It appears quite possible that the superior ranks held by the White Leghorns in most flocks may have been in part a function of the effects of the presence of two hens of this breed.

The results of pair-contacts between hens of different breeds vary somewhat from those of Collias (1943). In encounters between his White Leghorns, 78 per cent were won by the bird initiating the encounter, and 33.5 per cent of the encounters resulted in active fights. In initial encounters between hens of different breeds which were members of multiple-breed flocks, 98.6 per cent were won by the hen beginning the encounter and 19.5 per cent of all encounters resulted in active fights. Similarly, when hens were members of the single-breed flocks, 92.4 per cent of all staged encounters were won by the initiator, and 18.2 per cent resulted in active fights. Collias' figures for all-White Leghorn encounters were in conformity with results obtained from similar contacts staged between our White Leghorns preliminary to the present study. The discrepancies between observations of pair-contacts between hens of different breeds and between White Leghorn hens are probably a result of the greater differences in dominance which exist between birds of unlike breed and birds of the same strain of one breed. The data indicate that the dominant hen of a pair of strangers has the shorter latent period for an overt threat directed toward its opponent. (The initiator of an encounter usually wins it.) If dominance is a function of aggressive behavior, the response of the threatened bird (active resistance or passive submission) would be in part a function of its own dominance. Collias used White Leghorns of the same strain as were used in this study. The average level of dominance in his population was probably considerably higher than in the present population of seven breeds, six of which were observed to be less dominant than the White Leghorn.

Factors which affect dominance in hens have not been completely analyzed. In particular, little is known of the effects of various environmental conditions. Collias (1943) worked with measurable indices of physiological and psychological states of successful hens and was able to analyze only 56 per cent of the factors involved. Among those known physiologi-

cal and psychological factors which might have contributed to the observed differences in dominance between hens of the various breeds used, none seems to be of sufficient consequence to explain fully the differences in success. Conditioning may have played some role in emphasizing natural differences in dominance, but the consistent similarities in success of mature hens of the same breed, whether experienced or inexperienced, indicates that this was not a major factor in the present series of tests. Differences in dominance between hens of the given strains of different breeds appears to be associated with their different genetic backgrounds.

SUMMARY

1. The dominance relations of mature hens of the following seven breeds were observed in twelve flocks: White Leghorn, Brown Red Game, Rhode Island Red, White Wyandotte, Brown Leghorn, Light Brahma, and White Cochin Bantam. Statistically, White Leghorns were the most dominant of the tested hens, White Cochin Bantams the least.

2. The dominance scores of the intermediate breeds showed some overlap. Although no significant difference existed between Game hens and Rhode Island Reds, the former were significantly more dominant than the White Wyandottes or Brown Leghorns, and Rhode Island Reds were not. The Rhode Island Reds were, in turn, more dominant than Light Brahmas, whereas White Wyandottes, Brown Leghorns, and Light Brahmas were essentially equivalent in dominance.

3. Since hens of the same breed were usually obtained from the same source, they were, presumably, of the same strain of the given breed. Therefore, any genetic differences in dominance can be interpreted only as differences between given strains of the tested breeds and are not necessarily typical of the breeds in general.

4. Each hen was assigned a statistically adjusted rank based upon the number of its subordinates and the number of birds in its flock. An analysis of variance carried out with these data indicated that there were real differences between the ranks of the various breeds.

5. A total of 834 decisive initial encounters was staged in neutral pens between hens of the different breeds. The agreement between dominance in flocks and dominance won in initial encounters for seventeen of the twenty-one possible pairs of breeds indicated that these two types of samples were drawn from the same statistical universe. The four exceptional pairs were Rhode Island Red–Game, Rhode Island Red–Brown Leghorn, Rhode Island Red–Light Brahma, and Game–Brown Leghorn.

6. Factors known to influence the achievement of dominance in tests between hens of the same breed did not fully account for differences in dominance between hens of different breeds.

7. The published material relating to social behavior of the domestic fowl indicates that these birds recognize one another as individuals. Some observations reported in the present paper indicate that a hen's association with known individuals of various breeds may influence her behavior toward strangers of these breeds. Under certain conditions the hen appears to respond, at least partially, to the stranger's breed rather than to the stranger as an individual.

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NEW BOOKS IN PHYSIOLOGICAL ZOÖLOGY

[This section comprises reviews of selected new books containing new and significant material in the field of physiological zoölogy. Books submitted for review should be sent directly to the review editor: Frank A. Brown, Jr., Department of Zoölogy, Northwestern University, Evanston, Illinois.]

SYMPOSIA OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY, NO. II: *GROWTH IN RELATION TO DIFFERENTIATION AND MORPHOGENESIS*.[1]

Reviewed by JAMES W. BUCHANAN
Northwestern University

THIS volume contains most of the papers read at a symposium of the British Society for Experimental Biology, held at Cambridge in July, 1947. Nineteen are included, as follows: "The Role of the Cell in Determination," by V. G. Wigglesworth; "Concepts of Embryonic Induction and Their Relation to Parthenogenesis and Malignancy," by J. Holtfreter; "On the Developmental Physiology of the Sea Urchin," by S. Hörstadius and T. Gustafson; "Growth and Differentiation of Nerve Fibres," by J. Z. Young; "The Control of Flowering in Plants," by F. G. Gregory; "Fac-

[1] Cambridge: Cambridge University Press; New York: Academic Press, Inc., 1947. Pp. vii+365. $7.50.

tors Governing the Induction of Development of Reproductive Structures in Plants," by K. C. Hamner; "Vegetation and Reproductive Development of *Kalanchoë blossfeldianaas* Influenced by Photoperiodism," by R. Harder; "Growth, Differentiation, and Function of Explants of Some Endocrine Glands," by P. J. Gaillard; "The Genetic Control of Development," by C. H. Waddington; "Genes and Pathological Development in Mammals," by H. Grüneberg; "Gene Action in Growth and Differentiation of Lethal Mutants of Drosophila," by E. Hadorn; "Nucleus and Cytoplasm in Differentiation," by K. Mather; "The Geometry of Phyllotaxis and Its Origin," by F. J. Richards; "Differentiation in the Primary Shoots of *Lupinus albus L.*, and of *Tropaeolum majus* L.," by E. Ball; "On the Determination of Leaves," by Mary Snow and R. Snow; "Experimental Morphology with Special References to Pteridophytes," by C. W. Wardlaw; "Differentiation as the Controlled Production of Unique Enzymatic Patterns," by S. Spiegelman; "Morphogenic Factors as Exemplified by the Onion Plant," by O. V. S. Heath and M. Holdsworth; "Observations of the Present State of Embryology," by J. H. Woodger.

The distinction of this list of authors assures the high quality of the volume, even though the papers are uneven in weight and so diverse in subject matter that they may be said to be held together solely by the title of the volume.

Wigglesworth gives an excellent but very brief review of the role of the epidermis in morphogenesis in insects. He interprets the evidence to mean that control is mediated through chemical agents, distant differentiations being initiated and controlled from centers from which originate substances which suppress the latent potencies of adjacent regions to develop into like tissues. At points within this area of suppression new chemical changes take place which lead to the determination of other subsidiary parts. At this point Wigglesworth becomes somewhat unclear. The reviewer finds nothing to account for the origin of such centers except the author's statement that they arise in a self-determining system. Moreover, the role of morphogenic hormones and of genes is merely asserted as factors in morphogenesis.

Holtfreter gives a succinct review of the problem of embryonic induction, with particular reference to the amphibian organizer. Since much evidence renders almost certain the interpretation of the organizing agent as chemical in character, much investigation is concerned with the properties of such agents. Holtfreter searches for the common denominator of the many and varied agents and operations that are effective in various degrees as organizers. This portion of his paper is reminiscent of the searches by earlier workers for the common property of the very considerable variety of substances and conditions that will induce artificial parthenogenesis, of the search for the common agent in the experimental production of monstrosities, and of other incidents in the history of experimental zoölogy. The activability of the target cells by conditions which seem to be largely quantitative in character seems not to have been sufficiently studied.

Hörstadius and Gustafson contribute a short article dealing with a most promising area of investigation, namely, the specific nature of the enzymes that have to do with differentiation in the embryo of the sea urchin. Young's paper on nerve fiber regeneration, while detailed and valuable, is not easily brought into an area common to the preceding papers. Gregory's article on the control of flowering in plants and that of Hamner on the induction and development of reproductive structures in plants, together with Harder's on the influence of photoperiodism on reproduction in a specific plant, constitute an interesting and informative treatise which forms a substantial unit in the whole symposium. Here exogenous factors in development are treated, as compared with the endogenous influences operating in animal development and reviewed in the earlier papers in the volume. One wishes that Hamner had been somewhat more fastidious in some of his expressions. To read repeatedly of "exposure to darkness" is to put the reviewer's teeth slightly on edge.

Gaillard's paper on growth and differentiation of endocrine glands, while important, is a sort of aside in the major trends in the symposium. Waddington, however, returns to the principal theme in his paper on the genetic control of development. This is a serious attempt to integrate gene control with the chemical events of development. It is suggested that the gene influence may be mediated through plasmogenes, which, while partially independent of nuclear genes, do not exist in their absence. Grüneberg makes a further contribution to the subject in his review of the role of genes in pathological development in mammals. But Grüneberg's paper is too short to cover ade-

quately the facts that are known, together with the numerous possibilities that inevitably suggest themselves. Hadorn on the lethal mutants of *Drosophila* introduces more complexities into the already complex situation developed by the preceding authors in the volume, showing that various lethals exert their effects at various and obviously critical stages in development. Reading from right to left, this means that, inversely, the development processes are, step by step, positively under control of the gene complex of the sygote. Mather's paper on nucleus and cytoplasm in differentiation is in reality an essay that extends into broader areas the concept of gene control in development.

This reviewer does not feel competent to discuss Richard's article on the geometry of phyllotaxis and its origin, since it contributes a rather abstruse mathematical analysis of certain phases of form development in plants. Ball's article on the differentiation of the primary shoots of *Lupinus* and *Tropaeolum* returns to the general theme of the volume and includes an experimental analysis of the regulation of growth and differentiation, particularly by the method of explants. "On the Determination of Leaves," by M. and R. Snow, takes firmly to task the proponents of the "repulsion theory" and proposes in its place a mechanical interpretation of the determination of the site of development of the new leaf. There appears to be no room in the Snow interpretation of "first available space" for an organizer or induction.

Wardlaw contributes a brief paper on experimental morphology with special reference to Pteridophytes. The paper is general in character and emphasizes an interpretation of apicobasal developmental patterns as derived from or associated with apicobasal flow of auxins and other substances. Spiegelman contributes an extensive and highly suggestive treatment of differentiation considered as a consequence of unique enzyme patterns. In the present state of the problem he is confronted with many unknowns: the number of enzyme systems involved, their relation to one another, and the rise of a widening stream of enzymes and enzyme activities derived from earlier systems. He believes, however, that these possibilities find support in the facts known concerning genes and plasmogenes. This chapter alone is sufficiently provocative and stimulating to justify the entire volume. The last chapter dealing with a specific subject material is by Heath and Holdsworth and has to do with bulbing in the onion plant. It should have been placed in the section of the book with the chapters by Gregory, Hamner, and others; for it concerns itself with environmental controls of the morphogenesis of the whole plant as well as bulbing. The last chapter, by Woodger, is a somewhat loosely constructed treatment of the semantics of embryology, with an appeal for new hypotheses and a revised language. The reviewer is not in sympathy with Woodger's proposal that a small committee be formed, permanent in character, devoted to the search for key hypotheses. Fundamental Science is an art; its hypotheses, true or false, arise first in a single intellect and may not be successfully socialized.

This is a good book. Packed within its 365 pages is an imposing condensation of much material reviewing authoritatively the present status of some of the most important problems of all biology.

ERRATUM

Figure 24 of the paper, "Differential Intracellular Oxidation and Reduction of Indicators in Relation to Fission," by C. M. Child, is reprinted herewith. Through a printer's error the figure in *Physiological Zoölogy*, XXII, No. 2 (1949), 109, shows the short line representing the anal segment wrongly placed.

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